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RESEARCH PAPER

Effect of different fertilizers and sowing date on growth, yield, and yield components of Sesame *(Sesame indicum L.)*

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A B S T R A C T:

A factorial experiment was conducted on the summer season of 2019 at two locations Grdarasha experimental field (Latitude: 36° 4' N and Longitude: 44° 2' E- elevation 415 meters above Sea level), College of Agricultural Engineering sciences, Salahaddin University, Erbil Iraqi Kurdistan Region and Erbil Research Director field /Ainkawa (Latitude :' 36° 14' N, 43° 59 E, 420 meters above sea level) using factorial experiment arranged in randomized design to investigate the effect of Four different formula of fertilizers (NPK; NPK+ Magnesium and Micro elements (Zn, Fe); Magnesium and Micro elements (Zn, Fe); No fertilizer were added considered as control) on growth, yield and yield component of sesame crop (*Sesame indicum* L.) under three sowing dates (20th of May, 4th of June and 19th of June). Results showed a significant rapid increase of branches number per plant, number of capsules per plant, and number of seeds per capsule, 1000 seeds weight (g), biological yield, grain and straw yield (t ha^{-1}) when treated with (NPK plus Magnesium and Micro elements) fertilizer compared to the control treatment at the first sowing date. An interaction between first sowing date and second Fertilization formula recorded highest value compared to the others treatments.

KEY WORDS: fertilization, sowing date, Micronutrients, Sesame yield
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1. INTRODUCTION:

Sesame (*Sesamum indicum* L.) is belonging to Pedaliaceae family is regarded as one of the most important edible oil seed crops in the world because of its high oil content (45-60%), protein 20-25% and carbohydrates 15% and vitamins. Sesame seeds are involved in sweet Manufacturing, pastries and sprinklers in addition to use of its pallet in animal diets (Jan *et al*., 2014). Sesame considered one of the oldest crops in the world, science it cultivated for over 5000 years in all tropical and subtropical countries in Asia and Africa for its high-nutrition and edible seeds (Bisht *et al*., 1998). So, it called the Queen of oil seeds due to its virtue excellent quality and utility, (Hafiz and El-Bramawy, 2012).

From physiological view sesame is a short-day plant, drought- tolerant crop, requires adequate soil moisture for establishment and vegetative growth and development, (Olowe, 2007). The optimum temperature for growth range between 27–35 °C, temperatures below 20 °C inhibit germination and retard seedling's growth, (Bennett, 1995)

Fertilizers changed Sesame crop productivity and its industry has grown significantly, it was (Erman et al., 2011) reported that macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) are essential and important for plant growth and yield, (Erman *et al.*, 2011). (N P K) fertilizers have been extensively studied and proven to significantly intensify sesame yield in the tropics,

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while growth and yield of sesame were greatly influenced by the application of N fertilizer (Zenawi and Mizan, 2019). Micronutrients are essential for plant life and have an important role in crop development, so lack of any one of the micronutrients in the soil will cause limited growth. The beneficial effect of micronutrients may be attributed to its role in activating different enzymes in plants, the efficient utilization of applied nutrients improves and increases growth and yield components (Tiwari *et al*., 1996) and (Shanker *et al*., 1999). In the Iraq Kurdistan Region, sesame crop is cultivated and usually fertilized with nitrogen and phosphorus fertilizers, and no one gave ant attention to fertilize sesame with macro and micronutrients. There for this was done to investigate the effect of different fertilizers nitrogen, phosphorus, potassium, magnesium and two microelements (Iron and Zinc) with three different sowing dates on sesame growth, yield, and yield component in two different locations.

2. Materials and Methods

2.1. Studied Sites description: The experiment was conducted in two locations, Grdarasha field (Latitude: 36° 4' N and Longitude: 44° 2' E-elevation 415 Meters above sea level) The experimental researches field College of Agriculture Engineering Sciences, Salahaddin University Erbil Kurdistan Region of Iraq, and Erbil Research Directorial field /Ainkawa (Latitude:' 36° 14' N, 43° 59 E, 420 meters above sea level), during 2019 agricultural season. Some soil physiochemical properties for both locations were determined in table (1).

2.2. Experimental design: A factorial experiment was carried out in a randomized complete block design (RCBD) arrangement with three replications. The studied factors were sowing dates (20th of May, 4th of June, and 19th of June in 2019) and four different mineral fertilizers: Only (NPK); NPK + Magnesium and Microelements (Iron - Zinc); Magnesium and micro elements (Iron -Zinc); and no fertilization regarded as (control). The plot size was 9 m 2 (3 x 3 m) keeping one meter between plots. Each plot consisted of 4 rows having 60 cm row-to-row distance, and 30 cm within plants.

2.3. Agronomical practices: Experimental plots were prepared by dries ploughing the land twice vertically one on the other, the land leveling was done, then rows were established by chisel plow, after that, it was hand seeded with sesame (Somar) genotype on (20th of May, 4th of June, and 19th of June in 2019) with rate (60 kg ha^{-1}). Seeds were sown at 1-2 cm depth. The irrigation process was done by drip irrigation, other practices were done out when needed.

2.4. Fertilization type: Nitrogen formula urea 46%N was applied in two doses (80 kg N ha^{-1}) the first dose at sowing time and the second dose was applied 45 days after sowing (DAS). Phosphorus formula triple super phosphate 46% p_2o_5 was applied at the rate of (80 kg ha^{-1}) with sowing seeds. While liquid potassium (5L ha^{-1}) with concentration 50% k_2o was added in two doses (40 and 65) days after sowing (DAS). liquid Magnesium ($Mgso_4$) Dose was (5L ha^{-1}) was applied thrice (40, 65and 90) days after sowing. Iron ($Feso_4$) (3.5L. ha^{-1}) thrice (40,65and 90) days after sowing. Zinc ($Znso_4$) (2L ha^{-1}) three times (40,65 and 90) days after sowing. liquid fertilizer sprayed with 16L capacity knapsack sprayer after calibration to ensure the rate of 2000L ha^{-1}.

2.5. Recorded Data - recorded parameters in this study could be categorized to:

2.5.1. Vegetative growth characteristics

Plant height (cm) calculated from the soil surface to the top of the plant, the number of branches per plant recorded the number of branches in one plant.

2.5.2. Yield and yield components

Number of capsules per plant, number of seed in a capsule per plant, weights of 1000-grains (g), biological yield t ha^{-1}, economical yield t ha^{-1}, straw yield t ha^{-1} and harvest index (HI%) were recorded after harvesting (Jahan *et al*., 2019).

Biological yield = grain yield + straw yield -----1

$$\text{Harvest index \%} = \frac{\text{Grain yield}}{\text{biological yield}} * 100 \text{ (Dobermann, 2007)------2}$$

2**.6. Statistical analysis**: All recorded data were subjected to standard analysis of variance and means were compared using Duncan Multiple Range Test (DMRT) at 5% of probability using SPSS computer analysis version 22 according to (Bah, 2001)

3. Results & Discussion

3.1. The effect different mineral fertilization and sowing date on some growth and yield characteristics of (*Sesame indicum* L.) at Grdarasha and Ankawa locations**.**

3.1.1-Plant height:

Table (4and 5) shows the effect types of fertilizers on plant height was significant, science (NPK + Magnesium and Micro elements) treatment recorded maximum average mean values of plant high (143.01 and 111.89) cm respectively in both locations Grdarasha and Ainkawa. While minimum average mean values recorded under control plots in both locations were (126.28 and 102.89) cm respectively. (Asl, 2017) reported that nitrogen and phosphorus application may increase plant height, also these results with those found by (Jadav *et al*., 2010) and (Mahdi, 2014). Nitrogen promotes healthy growth in plants, vice versa deficiency of nitrogen decreased plant height, also low phosphorus concentration causes plant dwarfing. Zinc plays an important role in growth, hormone production and internode elongation, because it has a key role of many enzymes and proteins activation. Iron has an important role in the activation of meristematic cells and division hence elongation inter nodes (Alloway, 2008).

Combination NPK, Magnesium and Micro elements increased plant high compared to control plots. Plant height increased significantly at second sowing date compared to first and third sowing dates since registries the tallest height average mean values (138.96 and 116.75) cm in both locations respectively, while last sowing date recorded lowest plant height (128.24 and 98.25) cm in both locations respectively. These results are similar to what had been reported by (Al-Solagh, 2007). These variations in the results between sowing dates may be due to the environmental conditions that prevailed after sowing date, that were appropriate for the division and elongation of stem cells that may provide nutrients for effective plant growth areas in the plant (meristematic cells). An interaction between fertilization and sowing dates exhibited significant effects on plant height, where maximum value recorded with fertilization (NPK + Magnesium and Micro elements(Iron-Zinc) at second sowing date in both locations with values (149.90 and 121.00) cm, while minimum values recorded in control plots with last sowing date (122.67 and 95.00) cm at both locations.

3.1.2-Number of branches plant^{-1}

The Results in tables (4 and 5) shows in Grdarasha, sowing dates significantly influenced branches per plant in which first sowing date recorded maximum Average mean value (3.20 branches plant^{-1}), while minimum value was (1.58 branches plant^{-1}) registered in last sowing date. Similar results have been reported by (Mahdi *et al*., 2007). these results are going with those reported by. (Al-Solagh, 2007). The results regarding interaction between fertilization and sowing date significant effects appeared with (NPK) fertilizers in first sowing date, which recorded maximum value (3.86 branches plant^{-1}) while minimum value recorded in Magnesium and Micro elements plots when seeds were sown on last sowing date (1.27 branches plant^{-1})

3.1.3-Number of capsules plant^{-1}:

Data in tables (6 and 7) revealed that fertilization with (NPK + Magnesium and Micro- elements) significantly enhanced capsule number per plant since the highest average mean values were (100.42 capsules Plant^{-1} and 72. 56capsules plant^{-1}) in Grdarasha and Ainkawa locations. Whereas minimum Average mean values were (78.71 capsules plant^{-1}and 56.78 capsules plant^{-1}) recorded in control plots for both locations Grdarasha and Ainkawa respectively. According to (Jan *et al*., 2014; Ibrahim *et a*l., 2016; Mahdi, 2014 and Al-Maliky, 2015) number of capsules per plant were influenced by nitrogen, phosphorous and potassium fertilizer levels.

The role of potassium may enhance biological processes inside plants, increasing enzymatic activity and exchange of nutrients between cells. Nitrogen has a role in increasing the vegetative growth and thus increasing the process of photosynthesis. The lack of Fe means lack chlorophyll that decreases photosynthesis and unable of food production this led to decreases of capsule numbers in plant especially at flowering

stage. Zinc decreases photosynthesis rate and reduces the rate of plant components production, rather than the magnesium have a key role in chlorophyll chemical structure. Early sowing date on the 20th of may recorded higher capsule number with average mean values (110.28 capsules plant^{-1} and 70.67 capsules plant^{-1}) in both locations respectively. While the lower average mean number of capsules per plant was obtained in last sowing dates (67.39 capsules plant^{-1} and 62.76 capsules plant^{-1}) in both locations respectively Grdarasha and Ainkawa. These results are in agreement with (Al-Solagh, 2007). The reason for that may be due to the early sowing date of crop which had efficiency (photoperiod, temperature and humidity) For plant growth and development especially during the seed capsule formation which was positively reflected by photosynthesis rate.

Combination effect of between fertilization with (NPK + Magnesium and Microelements) and sowing date (first sowing date) recorded higher number of capsules with (129.44 capsules plant^{-1} and 80.00 capsules plant^{-1}) in both locations. When NPK fertilizers in combination with last sowing date obtained minimum number of capsules per plant which was (62.10 capsules plant^{-1}) in Grdarasha location, while in Ainkawa the minimum value recorded by NPK combined with first sowing date was (55.00 capsules plant^{-1}).

3.1.4-Number seeds per capsule:

The significant effect of fertilizers registered maximum mean number of seeds per capsule were observed in (NPK+ Magnesium and Microelements) treatment plots (73.35 and 72.74 seeds capsule^{-1}) for both locations respectively (table 8 and 9), while minimum average mean values were recorded in control plots (59.36 and 61.11seeds capsules. plant $^{-1}$) respectively in both locations Grdarasha and Ainkawa. These results are in line with (Buriro *et al*., 2015) and (Mahdi, 2014). These may refer to leaf production, high nitrogen use, higher leaves efficiency for seed assimilates (Malik et al., 2003). Activating many enzymes that increases the efficiency of the carbon metabolism processes (kobraee and Rasekhi, 2011). The highest average mean number of seeds per capsule was produced in early sowing (71.81 and 70.67 seeds. Capsule^{-1}) and lowest number of seed capsule per plant were obtained in late sowing date (64.86 and 62.76 seeds capsule-^{1}) respectively in both locations Grdarasha and Ainkawa, the results reported with (Fadil, 2015).

The declined seeds number in capsules at the last sowing dates may be attributed to the short period of growth compared to other sowing dates and the low temperature at the time of seed formation and filling, which negatively affected the photosynthesis process and its efficiency in preparing the emerging seeds with its requirements for growth and thus causes to a large number of seeds. Relying on interaction effect between fertilization and sowing date for these characters showed by (NPK + Magnesium and Microelements) treatment in first sowing dates which recorded maximum value (77.48 and 81.22) seeds capsule^{-1} in both locations Grdarasha and Ainkawa. Meanwhile the minimum values were observed by control with last sowing date plots which reached (57.00 and 60.00) seeds capsule^{-1} Grdarasha and Ainkawa.

3.1.5- 1000-thousand seeds weight (g):

(NPK+ Magnesium and Microelements) application caused significant effects on thousand seeds weight (table 10 and 11) when recorded maximum average mean values (3.67and 3.56) g in both locations Grdarasha and Ainkawa respectively. However, the control plots recorded the lowest average mean values were (3.37and 3.34) g respectively in both locations. The present results are also in line with those reported by (Akhtar *et al.*, 2015)

the effect of nitrogen and phosphorus on Sesame crop. These results are in accordance with those have been reported by (Shimgabr *et a*l., 2019; (Al-Maliky, 2015; Seervi *et al*., 2018 and Sarkar *et al*., 2007) reported that phosphorus encourages photosynthesis rate and the number of metabolites synthesized by plants. The role of potassium in elevating enzyme activity, especially the enzymes that induce compounds to synthesize with high molecular weights such as carbohydrates, sugars and starch, as well as have an important role in transfer and movement of carbohydrates from their places of formation to storage.

Depending on the effect of Zn and Fe on thousand seeds weight of sesame crop. It was found that an increasing in added Fe led to increase the accumulation of storge food in seeds, thus an increase their average mean weight, including starch. tables (10 and 11) indicated on to the significant effects of Fe and Zn on the weight of thousand seeds. Early sowing date recorded

highest value for the average mean of thousand seeds weight in both stations showed up (3.58 and 3.53) g in both locations respectively Grdarasha and Ainkawa. While lowest value in both locations shows up (3.41 g) in the third sowing date. These results are closely related to the findings of (Al-Solagh, 2007). The optimum sowing date has a great role in providing suitable conditions for effective photosynthesis, which means giving a suitable chance for better growth and thus better-accumulating nutrients, which is directly reflected in the rate of seed filling and weight. Data in tables (10 and 11) indicated that interaction between fertilization and sowing date recorded in (NPK+ Magnesium and Micro elements) in first sowing date, when the highest value reached (3.73 and 3.63) g while the lowest value recorded by control treatment in last sowing data reached (3.30 and 3.30) g in both locations respectively Grdarasha and Ainkawa.

3.1.6. Straw yield per plant (t ha^{-1})

The straw yield of sesame was also influenced significantly by different mineral fertilizers table (12 and 13). The highest data of average mean values were registered with (NPK + Magnesium and Microelements) which reach (4.15 and 3.55) t ha $^{-1}$ in both locations Grdarasha and Ainkawa. While the lowest values were recorded at control plots (3.77 and 3.09) t ha^{-1} for both places Grdarasha and Ainkawa. This result was in line with (Jahan *et al*., 2019) when they studied the effect of phosphorus on straw yield and (Bijarnia *et al*., 2019) when investigated the effect of nitrogen and potassium on crop. Sowing date caused significant influences on straw yield of Sesame, the highest average mean values of straw yield were (4.79 and 3.54) t ha^{-1} obtained from the first sowing date in both locations respectively. While, the lowest values were (3.55 and 3.18) t ha^{-1}recorded from last sowing date in both locations respectively. This result is in line with results was obtained by (Al-Solagh, 2007). The reason may be due to the long growth period or to the increase in the number of plant branches, and thus to the prolonged period of exposure to sunlight, that elevated synthesis materials which increases the dry weight of the dry plant .The combination effects between fertilization and sowing, at Gardarasha locations maximum value recorded at treatment (NPK + Magnesium and Microelements in first sowing date) was (5.09) t ha-^{1}, while minimum value recorded at Magnesium and Microelements in third sowing date which was (3.51) t ha^{-1}. While, at Ainkawa maximum value which was recorded at plot treatment (NPK + Magnesium and Microelements in second sowing date) was reached (3. 62t ha^{-1}). As usual minimum value recorded at control plots in last sowing date reached (2. 56t ha^{-1}).

3.1.7-Grain yield per plant (t ha^{-1}):

Data regarding grain yield are shown in table (14 and 15) which indicated to the significant effect of fertilization on this trait. The highest average mean values were (2.40 and 2.07) t ha^{-1} recorded with (NPK +Magnesium and Microelements) contrary to control that recorded lowest value (2.10 and 1.58)t ha^{-1}in both locations respectively Grdarasha and Ainkawa. These results were consistent with (Hadif, 2012; Haruna *et al*., 2012; Amare *et al*., 2019 and Thakur *et al*., 1998). Greater average mean of grain yield was recorded by planting sesame on the 20th of May (2.40 and 2.16) t ha^{-1} in both locations respectively Grdarasha and Ainkawa. However, second sowing date takes the same letter a in both locations. While lowest value of average mean of grain yield recorded on 19th of June reached (2.07 and 1.68) t ha^{-1}in both locations respectively Grdarasha and Ainkawa. Similar results were studied by (Fadil, 2015). It appears from data presented in table (14 and15) that the interaction effects between first sowing date and second formula of fertilization recorded highest value reached (2.55 and 2.59) t ha^{-1} in both locations respectively Grdarasha and Ainkawa. While lowest values were recorded by control plots in last sowing date (1.98 and 1.53) t ha^{-1} in both locations respectively Grdarasha and Ainkawa. These findings were similar to results obtained by (Hafiz and El-Bramawy, 2012) when studied the interaction between phosphorus and potassium Sesame (*Sesame indicum* L.).

3.1.8-Biological yield (t ha^{-1})

Biological yield significantly responded to mineral fertilizers (NPK+ Magnesium and Micro elements) application which recorded highest average mean values (6.55and 5.62) t ha^{1} in both locations Grdarasha and Ainkawa respectively. Control plots recorded lowest data (5.87and 4.67) t ha^{1} in both locations respectively table (16 and 17). Early sowing date significantly improved biological yield; the highest average mean values of biological yield was recorded when the crop

was sown on 20th of May (7.19 and 5.59) t ha^{-1}in both locations respectively. Contrast to that 19th of June sowing date recorded lowest biological yield reached (5.62 t $ha^{-1}$and 4.86 t ha^{-1}) in both locations respectively. These results are opposite what has been reported by (Hakeem *et al*., 2017). The combined impact of fertilization and sowing date were significantly affected on biological field at interaction between (NPK +Magnesium and Microelements) when interact with first sowing date, since the plants obtained maximum values (7.64 and 6.09) t ha^{-1} in both locations respectively Grdarasha and Ainkawa. While their Minimum values of biological yield were recorded at control plot in last sowing date reached (5.4 and 4.9) t ha^{-1} in both locations respectively Grdarasha and Ainkawa.

3.1.9-Harvest index%

Table (18) shows the significant effect of fertilization types on the harvest index at Ainkawa which (Akhtar *et al*., 2015)average mean value was (0.37%) that recorded with (NPK +Magnesium and Microelements) fertilization, while lowest value was recorded at control plots (0.34%) however micro elements plot take same letter. These results are in close conformity with the findings with (Bijarnia *et al*., 2019; Heidari *et al*., 2011; Seervi *et al*., 2018). Significant differences were found for the average mean value for the sowing dates in case of its effect on harvest index. Under Grdarasha environment second sowing date gained maximum value (0.39%) while minimum value was registered with first sowing date (0.33%:). At Ainkawa location maximum average mean value recorded with first sowing date (0.38%) while minimum value recorded at second sowing date (0.32%). Significant different were found in Ainkawa, when higher value was recorded the interaction effect between (NPK +Magnesium and Microelements) plots with first sowing date (0.43%) while minimum value obtained at control in second sowing date (0.32%).

4. Conclusions

Types of fertilizer and sowing date are important factors that enhances vegetative growth, yield and yield components of sesame in both studied locations, best fertilization type was combination between (NPK +Magnesium and Microelements) and best sowing date was first sowing date for both locations.

Table (1): Some physical and chemical properties of the soil used in the Ainkawa and Grdarasha experiment

#	Locations	EC (dSm^{-1})	pH	N %	P (ppm)	K (ppm)	O.M %	Classification USDA			
								Clay%	Silt%	Sand%	Texture
1	**Ainkawa.**	0.3	7.9	0.09	7.86	180	1.0	38	43	19	Silty clay loam
2	**Grdarasha.**	0.3	7.3	0.14	9.7	300	1.5	48.4	43.3	8.3	Silty clay

The Soil properties were analyzed in Agriculture Research Centre - Ainkawa /Erbil.

Table (2): effects type of fertilizer and sowing date on plant high of *Sesame indicum* L. (Grdarasha).

FSD	F1(NPK)	F2(NPK+ (Mg+ Microelements	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	129.83ab	145.30ab	143.43ab	125.67ab	136.06ab
2nd SD	135.12ab	149.90a	140.33ab	130.50ab	138.96a
3rd SD	125.53ab	133.83ab	130.93bc	122.67b	128.24b
Mean	130.16bc	143.01a	138.23ab	126.28c	134.42

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (3): effects type of fertilizer and sowing date on plant high of *Sesame indicum* L. (Ainkawa)

FSD	F1(NPK)	F2 (NPK+ Mg+Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	107.67cde	112.67abc	110.00bcd	105.00cdef	108.83b
2nd SD	118.00ab	121.00a	119.33a	108.67cd	116.75a
3rd SD	97.00fg	102.00defg	99.00efg	95.00g	98.25c
Mean	107.55B	111.89a	109.44ab	102.89c	107.94

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (4): effects type of fertilizer and sowing date on number of branches of *Sesame indicum* L. (Grdarasha).

FSD	F1(NPK)	F2(NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	3.86a	3.28ab	3.33ab	2.33abc	3.20a
2nd SD	2.33abc	2.34abc	2.50abc	1.90bc	2.27b
3rd SD	1.70bc	2.00bc	1.27c	1.35c	1.58c
Mean	2.63a	2.54a	2.37a	1.86a	2.35

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (5): effects type of fertilizer and sowing date on number of branches of *Sesame indicum* L. (Ainkawa).

FSD	F1(NPK)	F2 (NPK+ (Mg+ Microelements)	F3(Mg+ Micro elements)	F4(control)	Mean
1st SD	1.50a	1.67a	1.30a	1.33a	1.45a
2nd SD	1.30a	1.50a	1.10a	1.11a	1.25a
3rd SD	1.20a	1.27a	1.00a	1.00a	1.12a
Mean	1.33a	1.48a	1.13a	1.15a	1.27

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (6): effects type of fertilizer and sowing date on number of capsules per plant of *Sesame indicum* L. (Grdarasha).

FSD	F1(NPK)	F2(NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	96.39cd	129.44a	120.17ab	95.13cd	110.28a
2nd SD	82.34de	104.67bc	101.73c	74.00ef	90.69b
3rd SD	62.10f	67.16ef	73.30ef	67.00ef	67.39c
Mean	80.28b	100.42a	98.40a	78.71b	89.45

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan.

Table (7): effects type of fertilizer and sowing date on number of capsules per plant of *Sesame indicum* L. (Ainkawa).

FSD	F1(NPK)	F2 (NPK+ Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	74.00ab	80.00a	73.67abc	55.00g	70.67a
2nd SD	66.67cde	70.00bcd	64.00def	58.00fg	64.67b
3rd SD	66.00de	67.67cde	60.04efg	57.33fg	62.76b
Mean	68.89b	72.56a	65.90ab	56.78c	66.03

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (8): effects type of fertilizer and sowing date on number seed of capsules per plant of *Sesame indicum* L. (Grdarasha).

FSD	F1(NPK)	F2 NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	72.72ab	77.48a	76.00a	61.03bc	71.81a
2nd SD	69.08abc	72.78ab	70.00abc	60.06bc	67.98ab
3rd SD	65.83abc	69.78abc	66.83abc	57.00c	64.86b
Mean	69.21a	73.35a	70.94a	59.36b	68.21

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (9): effects type of fertilizer and sowing date on number seed of capsules per plant of *Sesame indicum* L. (Ainkawa).

FSD	F1(NPK)	F2 NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	69.67bcd	81.22a	71.17b	62.33cde	70.67a
2nd SD	67.67bcd	70.00bc	69.00bcd	61.00cd	64.67b
3rd SD	65.00bcd	67.00bcd	66.00bcd	60.00d	62.76b
Mean	67.44b	72.74a	68.72ab	61.11c	66.03

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (10): effects type of fertilizer and sowing date on 1000-thousand seeds weight of *Sesame indicum* L. (Grdarasha).

FSD	F1(NPK)	F2 NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control	Mean
1st SD	3.57ab	3.73a	3.53abc	3.47bc	3.58a
2nd SD	3.47bc	3.73a	3.43bc	3.33bc	3.49ab
3rd SD	3.43bc	3.53abc	3.37bc	3.30c	3.41b
Mean	3.49b	3.67a	3.44bc	3.37c	3.49

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (11): effects type of fertilizer and sowing date on 1000-thousand seeds weight of *Sesame indicum* L. (Ainkawa).

FSD	F1(NPK)	F2 (NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	3.57ab	3.63a	3.53ab	3.40ab	3.53a
2nd SD	3.50ab	3.53ab	3.50ab	3.33ab	3.47ab
3rd SD	3.44ab	3.50ab	3.40ab	3.30b	3.41b
Mean	3.50a	3.56a	3.48a	3.34b	3.47

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan.

Table (12): effects type of fertilizer and sowing date on straw yield of *Sesame indicum* L. (Grdarasha).

FSD	F1(NPK)	F2 (NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	4.81ab	5.09a	4.81ab	4.44abc	4.79a
2nd SD	3.63c	3.71ab	3.6c	3.43c	3.59b
3rd SD	3.61c	3.64abc	3.51c	3.44c	3.55b
Mean	4.02a	4.15a	3.97a	3.77a	3.97

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (13): effects of fertilization type and sowing date on straw yield of *Sesame indicum* L. (Ainkawa).

FSD	F1(NPK)	F2 NPK+ (Mg+ Microelements	F3 Mg+ Micro elements	F4(control)	Mean
1st SD	3.46abcd	3.50abcd	3.45abcd	3.33cd	3.43b
2nd SD	3.61ab	3.62a	3.53abc	3.38abcd	3.54a
3rd SD	3.24d	3.52abc	3.37bcd	2.56e	3.18c
Mean	3.45a	3.55a	3.45a	3.09b	3.38

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (14): effects type of fertilizer and sowing date on grain yield of *Sesame indicum* L. (Grdarasha).

FSD	F1(NPK)	F2 NPK+ (Mg+ Microelements	F3 Mg+ Micro elements	F4(control	Mean
1st SD	2.40ab	2.55a	2.44ab	2.22ab	2.40a
2nd SD	2.38ab	2.50a	2.44ab	2.11ab	2.36a
3rd SD	2.13ab	2.16ab	2b	1.98b	2.07b
Mean	2.30ab	2.40a	2.29ab	2.10b	2.27

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan.

Table (15): effects type of fertilizer and Sowing date on grain yield of *Sesame indicum* L. (Ainkawa).

FSD	F1(NPK)	F2 (NPK+ (Mg+ Microelements)	F3(Mg+ Micro elements)	F4(control)	Mean
1st SD	2.38b	2.59a	2.01c	1.66def	2.16a
2nd SD	1.8d	1.83cd	1.73d	1.55ef	1.73b
3rd SD	1.74def	1.79d	1.66def	1.53f	1.68b
Mean	1.97b	2.07a	1.80c	1.58d	1.85

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (16): effects type of fertilizer and sowing date on biological yield of *Sesame indicum* L. (Ainkawa)

FSD	F1(NPK)	F2(NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	7.21a	7.64a	7.25a	6.66ab	7.19a
2nd SD	6.01bc	6.21ab	6.04bc	5.54bc	5.95b
3rd SD	5.74bc	5.8bc	5.51c	5.42c	5.62c
Mean	6.32ab	6.55a	6.26ab	5.87b	6.25

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (17): effects type of fertilizer and sowing date on biological yield of *Sesame indicum* L. (Ainkawa)

FSD	F1(NPK)	F29(NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	5.84a	6.09a	5.46b	4.99de	5.59a
2nd SD	5.41b	5.45b	5.26bcd	4.93e	5.27b
3rd SD	4.98de	5.31bc	5.03cde	4.09f	4.86c
Mean	5.42b	5.62a	5.25c	4.67d	5.24

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan.

Table (18): effects type of fertilizer and sowing date on harvest index of *Sesame indicum* L. (Grdarasha)

FSD	F1(NPK)	F29 NPK+ (Mg+ Microelements)	F3 (Mg+ Micro elements)	F4(control)	Mean
1st SD	0.33%a	0.33%a	0.34%a	0.33%a	0.33%b
2nd SD	0.39%a	0.38%a	0.40%a	0.38%a	0.39%a
3rd SD	0.37%a	0.375%a	0.36%a	0.36%a	0.37%a
Mean	0.36%a	0.36%a	0.37%a	0.36%a	0.36%

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan

Table (19): effects type of fertilizer and sowing date on harvest index of *Sesame indicum* L. (Ainkawa)

FSD	F1 (NPK)	F1 (NPK+ Mg+ Microelements)	F3 (Mg+ Micro elements)	F4 (control)	Mean
1st SD	.41%ab	.43%a	.37%c	.33d	.38%a
2nd SD	.33%d	.33%d	.33%d	.32%d	.33%c
3rd SD	.35%cd	.34%d	.33%d	.38%bc	.35%b
Mean	0.36%a	0.37%a	0.34%b	0.34%b	o.35%

Note: Means with the same symbols in one column are not significantly different from each other at alpha = 0.05 based on multiple range test of Duncan.

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RESEARCH PAPER

Effect of Humic Acid on Tolerance indexes of Barley plant to Cadmium Toxicity

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A B S T R A C T:

This study was carried out to evaluate the effect of different levels of Humic acid (0, 400, 800 ppm) on tolerance index of barley plant to different levels (0, 25, 50 and 100 ppm) of Cadmium. The experiment was designed according factorial completely randomized design with three replications. The results revealed that the combination effect of Humic acid and Cadmium significantly increased the total dry weight, number of spikes and dry weight of straw, as well as in total dry weight of plant, cadmium tolerance was found by treatments HA0CD50, HA0CD10, HA400CD0, HA400CD25, HA400CD100, HA800CD50, HA800CD100. Cadmium significantly decreased the relative chlorophyll production rate (RCPR) of Barley plant,

Key worlds: Humic acid; Cadmium; Tolerance index; Barley plant.
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1. INTRODUCTION:

Barley (*Hordeum vulgaris* L.) is annual grass belong to gramineae family, which is a monocotyledonous herb, it is a short season, early found in varying environment (Gene technology regulator, 2017). It is an important cereal crop in the world after maize, wheat and rice. It is one of the earliest domesticated food crops since the start of civilization. Barley is cultivated in a wide range of environmental conditions (Wali *et al.*, 2018),), its used as animal food, and also for human, as well as used in industrial purposes for producing starch (Mantilla, 2015). Humic acid (HA) is natural organic compounds that can be used to increased nutrient availability, increase growth and yields. Humic acid produced commercially which is produced in decomposition of organic compounds.

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It contains nutrients that increased fertility of soil, availability of water and nutrients (Hamad and Tantawy, 2018). HA has indirectly many physiological roles in plants such as cell respiration, photosynthesis process, synthesis of proteins, the activity of enzymes which lead to growth and development of plants (Wali *et al.*, 2018). (Gomes Junior *et al.*, 2019) reported that HA increased uptake of nutrients such as phosphorus, magnesium, potassium, sodium in wheat plant, was particularly important for transportation and availability of micro nutrients under different environmental condition. Humic acid are well known as stimulators of plant germination and growth, which have anti-stress effects under abiotic stress condition such as unfavourable temperature, salinity, pH, etc. (Abdellatif *et al.*, 2017) mentioned that HA significantly increased plant height, fresh weight, number of flower clusters, number of flowers per plant and yield components such as fruit number per plant, fruit weight, total yield in tomato plant.

Heavy metals make a main source of environmental pollution as a result of human

activates such as energy and fuel production, power transmission, smelting, sludge damping, intensive agriculture (Jali *et al.,* 2016). Cadmium is one of the non-essential toxic elements to plants that transported from soil to plants by their roots, and accumulated in the roots, shoot, fruits and seeds. Accumulation of cadmium in plants cause phytotoxicity, inhibit growth and development of the plant, as well as effect on various physiological, morphological, biochemical activities of plant species like chlorosis, leaf rolls, necrosis, imbalance of mineral nutrients, variation of enzymes activity, photosynthetic rate, chrolophyll content (Liu *et al.,* 2014). The adverse effect of cadmium on growth of plant, exchange of gases and chlorophyll fluorescence decreased by application of humic acid in (*Orthosiphon stamineus)* plant (Ibrahim *et al.,* 2017). (Chaab *et al.,* 2016) found that humic acid has a great role in decrease the negative effect of cadmium on maize plant. (Konakci *et al.,* 2018) mentioned that Humic acid induced the toxicity of cadmium stress by changing photosynthetic apparatus and antioxidant activity in wheat leaves.

High tolerance of plants to heavy metals toxicity related to the ability of roots to tolerate Cd entrance as the toxic metal to the roots first, which make the plants to have a potential mechanism to metal tolerance such as cadmium stress (Azevedo *et al.,* 2012). (Venkataramaiah *et al.* 2011) showed that rice plant has several strategies to improve tolerance against heavy metal pollutant especially cadmium.

Rapid increase of industrialization and population growth at 15 last decade in Kurdistan region led to increase the environmental pollution particularly the soil pollution by heavy metals like Cadmium, thus the aim of this work was to study the assessment effect of foliar spray application of Humic acid on tolerance index of Barley plant to cadmium toxicity.

1. MATERIALS AND METHODS

1.1. Experimental design

A Pot experiment was conducted in glass house of biology department in the college of science- Salahaddin University-Ebil to study the effect of Humic acid on tolerance index of Barley plant cadmium toxicity. The experiment was involved 36 plastic pots with 24 cm diameter and 21 cm lenght, each pot was filled with 7kg of dried sandy loam soil collected from Aski kalak area, the soil sieved through 2mm pore size sieves. In each pot, five seeds were sown and then thinned to three plants later. Factorial experiment consisted of 12 treatment combinations of both Humic acid at does (0, 400, 800) ppm and soil irrigation containing (0, 25, 50 and 100)ppm cadmium replicated three times in completely randomized design (CRD) . Diammonium phosphate (DAP) Fertilizers containing 18% N and 64% P, were added to each pots.

At harvest the plants were cut at soil surface from each pots, placing them in weighted bag and oven dried at (70) ^{o}C for (48) hrs and after that the total dry weight, of thousand grain weight, straw weight, spike number, grain number, Relative growth rate (RGR), Relative chlorophyll rate (RCPR) were obtained.

The area of flag leaf (cm^2) was measured according by adopting stickler's linear measurement method (Stickler *et al.,* 1961).

Leaf area cm^2=L*B* 0.747

L= Length of leaf, B=width of leaf

Chlorophyll content in leaves was estimated by chlorophyll meter by clipping the sensor on different locations on leaf surface except the veins (Padilla *et al.,* 2015).

2.2. Statistical analysis

Analysis of variance was conducted to the obtained data and the treatment means were compared according to Duncans multiple range test at 0.05 and 0.01 level tests for greenhouse and laboratory parameters (Al-Rawi and Khalafulla, 1980). The statistical analysis was done by using Statistical Package for Social Sciences (SPSS version 26 software). For drawing graph, Excel 2010 software was used.

2. RESULTS AND DISCUSSION

Table (1) shows that HA800CD50 is superior significantly to some other treatment combinations with respect to total dry weight of Barley plants which indicates that higher concentration of HA causes the decreases of negative effect of Cadmium, these results agreed with those obtained by (Ibrahim *et al.,* 2017), who found that the humic acid application decrease the negative effect of cadmium on total biomass in *Orthosiphon stamineus* plant. HA

stimulate plant growth by increasing the chlorophyll content, uptake of nutrients as well as the adverse the effect of cadmium in Radish plant (Farouk *et al.,* 2011). These combinations also have significant effect of total dry weight at treatment (HA 800 CD50) as compared to control.

The results in table (2) show that combination effect of Humic acid and cadmium significantly increased the number of spike at treatment (HA 800 CD 50), as well as dry weight of straw at treatment(HA400 CD100) as compared to controls. Application of organic substance like humic acid enhance plant growth by increasing cell membrane permeability, respiration, oxygen and phosphorus uptake and increasing the growth of root cells (Chaab *et al.,* 2016).

Figure (2) show that cadmium significantly decreased the RCPR in barley plant at treatment (CD100) as compared to the control These results agree with those obtained by (Yang *et al.,* 2020), who found that the content of chlorophyll b and total chlorophyll decreased significantly with different concentration of cadmium in Davidia inolucrata plant. Chlorophyll content influenced by higher concentration of Cd. Cd might be accumulated by the plant and inhibit plant growth and other crops, nearly 40% of Cd may be absorbed and transported to the upper parts of the plant which directly affect the plants.(Abedi and Mojiri, 2020).

Fig (3) indicates that plants treated with (HA0CD25, HA400CD50, HA800CD0,HA800CD25)are non-tolerant (sensitive) plants to Cd toxicity as their tolerance indexes are negative according to the means of total dry weight compared to the control (HAOCDO) , while the rest of the plants of positive tolerance indexes considered tolerant to the toxicity. On the other hand, control plants are moderate. (Yasmin khan, *et al.,* 2017) revealed that HA enhanced shoot dry biomass as well as decreased the effect of Cd concentration in (*Brassica rapa)* plant. Foliar application of HA alleviating the symptoms of cd toxicity by increasing the content of photosynthetic pigments, root, shoot and leaf biomass in Hybrid *Pennisetum* (Song *et al.,* 2020).

HA successfully decreased the harmful effect of Cd stress by increasing total dry weight, number of spike and dry weight of straw.

Table 1: Combine effect of Humic acid and cadmium on some vegetative growth of Barley plant:

Treatments	Chlorophyll 1	Chlorophyll 2	First plant height(cm/plant)	Second plant height(cm/plant)	Flag leaf area ($cm^{2)}$	Total dry weight (gm)
HA 0 CD 0	53.96 ab	39.93 a	36.66 a	62.00 a	5.35 a	6.29 b
HA0 CD25	57.200 ab	49.36 a	41.16 a	63.00 a	7.27 a	3.69 b
HA 0CD 50	54.20 ab	47.36 a	43.00 a	74.33 a	5.47 a	8.79 ab
HA 0CD100	54.16 ab	48.73 a	39.66 a	70.00 a	9.99 a	8.30 ab
HA400CD0	56.16 ab	47.63 a	45.03 a	72.00 a	4.02 a	8.23 ab
HA400CD25	56.30 ab	51.43 a	44.33 a	67.66 a	5.94 a	7.043 ab
HA400CD50	56.56 ab	51.6 a	45.20 a	64.33 a	7.59 a	6.96 b
HA400CD100	50.70 b	53.43 a	39.53 a	62.33 a	4.14 a	8.53 ab
HA800CD0	53.66 ab	41.10 a	45.83 a	65.33 a	3.52 a	6.78 b
HA800CD25	56.20 ab	42.23 a	33.33 a	61.33 a	6.58 a	6.21 b
HA800CD50	59.00 a	53.66 a	43.66 a	65.66 a	8.78 a	12.26 a
HA800CD100	54.93 ab	48.23 a	53.83 a	69.66 a	6.31 a	8.18 ab

Table 2: Combine effect of Humic acid and cadmium on some yield characteristics of Barley plant:

Treatments	RCPR	Spike number /plant	RGR (g/g/days)	Dry weight of grains (gm)	Dry weight of straw(gm)	Thousand GW(gm)
HA 0 CD 0	0.0065 a	1.66 c	0.103 a	2.64 abc	0.59 cde	138.08 abc
HA0 CD25	0.0032 a	2.33 bc	0.0094 a	1.55 bc	0.18 e	88.07 bc
HA 0CD 50	0.0028 a	4.00 abc	0.0114 a	2.82 abc	0.64 bcde	147.50 abc
HA 0CD100	0.0021 a	2.66 bc	0.0123 a	3.29 ab	0.50 de	171.79 abc
HA400CD0	0.0033 a	3.00 bc	0.0095 a	2.96 abc	0.63 bcde	166.96 abc
HA400CD25	0.0018 a	2.33 ab	0.0094 a	3.61 ab	0.54 de	196.43 abc
HA400CD50	0.0020 a	2.66 bc	0.0084 a	2.45 abc	0.86 abcd	133.89 abc
HA400CD100	0.0011 a	4.66 ab	0.0092 a	4.33 a	1.18 a	270.41 a
HA800CD0	0.0070 a	3.00 bc	0.0079 a	4.00 a	1.13 abc	251.53 a
HA800CD25	0.0073 a	2.66 bc	0.0133 a	3.36 ab	0.66 bcde	218.54 ab
HA800CD50	0.0020 a	5.66 a	0.0094 a	1.15 c	1.16 ab	72.22 c
HA800CD100	0.0026 a	4.00 abc	0.0054 a	3.27 ab	0.86 abcd	212.35 ab

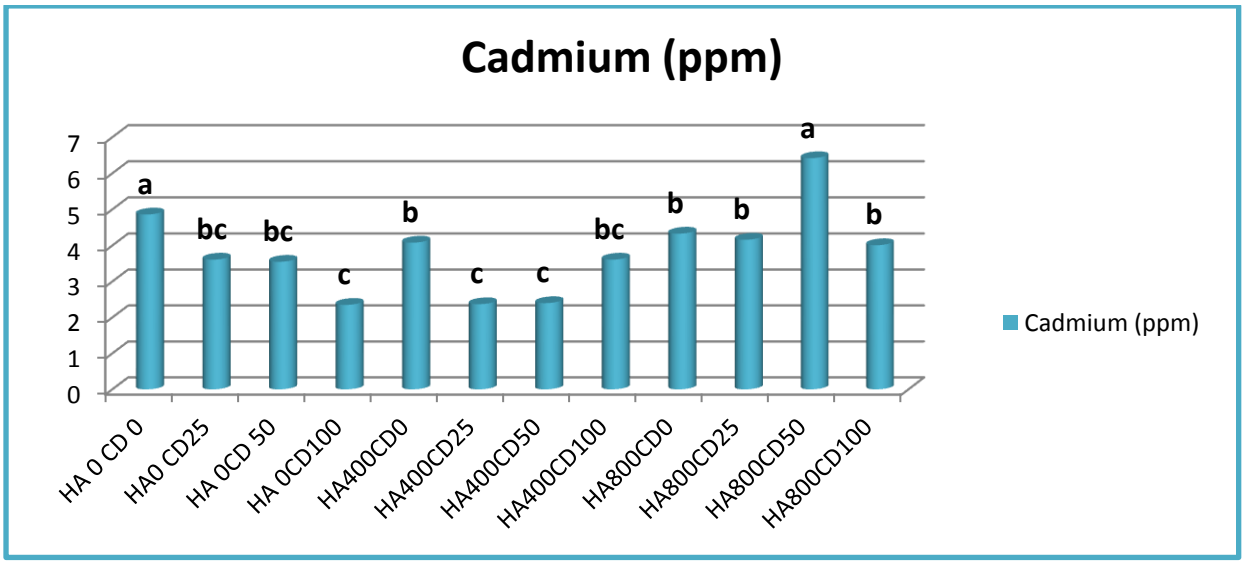

Fig 1: Combined effect of Humic acid and Cadmium on cadmium content of leaves in Barley plant.

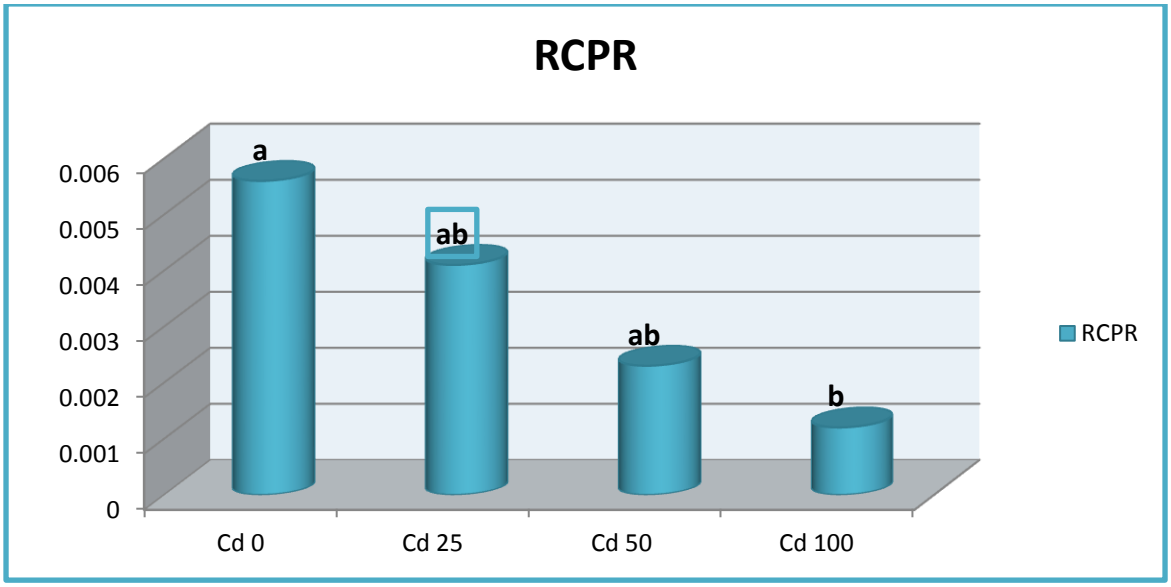

Fig 2: Effect of Cadmium on relative chlorophyll production rate in Barley plant.

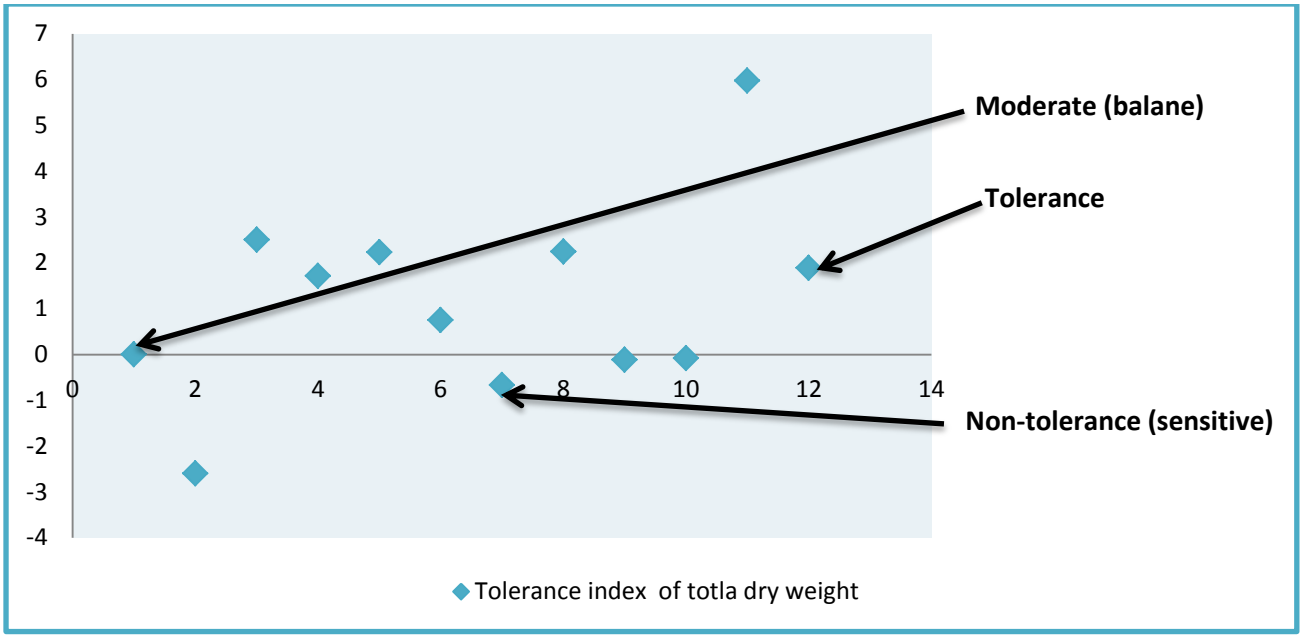

Fig 3: Combine effect of Humic acid and Cadmium on Tolerance index of total dry weight in Barley plant.

Conclusion

By the present study, the combine effect of Humic acid and Cadmium significantly increased the total dry weight, number of spikes and dry weight of straw, total dry weight of plant have tolerance. Although Cadmium significantly decreased the relative chlorophyll production rate (RCPR) of Barley plant. Humic acid has positive effect to decrease the cadmium effect in barley plant, thus suggested that Humic acid able to decrease the harmful effect of more heavy metals which has negative effect on plants and enviromrnt.

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RESEARCH PAPER

Propagation Characteristics of Electromagnetic Waves in Multilayered Biological Human Tissue

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A B S T R A C T:

This paper uses CST-2014 software package and Matlab 2018b to provide the data required to analyze the propagation mode characteristics of the electromagnetic waves (EM) inside multilayered tissue structure such as tissue layer impedance (Z) and tissue layers interface reflection coefficient (K). Moreover, the paper presents the distribution behavior of the electrical field (E-Field) and specific absorption rate (SAR) inside multilayered tissue.

The results show that the value of K is of the order of 0.7 at the air-skin interface, but it is about 0.1 at the tissue-tissue interface. The results also reveal that the SAR is directly proportioned to the frequency; in contrast, the standing wave (SW) of the E-filed is inversely proportioned with frequency.

Three multilayered phantoms (body-shell, thyroid, and testis) were adopted, and the results were obtained at three resonant frequencies over the ultra-wideband (UWB) frequency span of 3GHz, 6.5GHz, and 10GHz. The simulation results proof the computational formulas.

Key worlds: Electromagnetic Waves, Multilayered tissue, Human Organ phantom, reflection coefficient and standing wave ratio, specific absorption rate (SAR).
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1. INTRODUCTION:

In recent years, researchers have been interested in analyzing the interaction between the electromagnetic wave present in the atmosphere and human tissue. However, the human tissue is composed of multi-layers with different electrical properties which dependent on radio wave frequency (Mohammed and Saber, 2014).

The study of penetration performance of the electric field inside human organs is one of the important topics to be interested in order to estimate the values of the specific absorption rate (SAR). The SAR factor is essential for determining the amount of heat produced in human organs when exposed to electromagnetic waves as well as its importance in many detection and therapeutic applications such as CT scan and Hyperthermia unit (G. and E., 2019).

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Besides, it can provide health-monitoring services using body-worn devices (Genovesi, Butterworth and Daniel, 2018). On the other hand, the interactions between electromagnetic radiation and the living organism depend mostly on the quantity and characteristic of the transmitted energy with the kind of the exposed tissue (Adah *et al.*, 2018).

Moreover, some studies show a significant relationship between the amount of electromagnetic waves and the values of Thyroid‐Stimulating Hormone (TSH) for people without or with a family history of thyroid dysfunction (Velayutham, Sivan Arul Selvan and Unnikrishnan, 2015; Baby, Koshy and Mathew, 2017).

Other researches have demonstrated that continued exposure to high-frequency radiation by communication terminals may cause a decrease in testicle weight and size in mice. Where it

addressed the long term exposure of electromagnetic radiation could causes male infertility in mice.(Mugunthan *et al.*, 2015).

In the literature, considerable numbers of researchers discussed the interaction between the tissue and the electromagnetic waves.

(Adah *et al.*, 2018) presented some of the effects of electromagnetic radiation on the male reproductive system. It exhibits that Electromagnetic radiation impacts the male reproductive system causing a reduction in the mobility of spermatozoa, morphometric irregularity, and histological deviances in the testis and occasionally testicular tissue atrophy.

Y.E. Mohammed and Ali G. Saber (Mohammed and Saber, 2014), investigated the value of the electrical field with penetration depth into the phantom of the human tissue at different Medical Implant Communication System (MICS) and Industrial Scientific and Medical (ISM) frequency bands. They noted that when the penetration depth (δ) of the muscle greater than its thickness, the reflected signal will form a standing wave ratio. While if the penetration depth (δ) is smaller than its thickness, the exponential fading of the signal will occur inside the muscle.

S, Genovesi, I. Butterworth, and L. Daniel (Genovesi, Butterworth and Daniel, 2018), designed a matching layer (ML) that able to enhance the electric field penetration, they suggested Fat layer to be a ML and they tested for an imping signal with frequency interval (1.0-6.0) GHz. Their results showed that magnitude of the electric field entering the muscle layer is calculated for a fat layer thickness within (1.0-20) mm, also they observed that at lower frequencies (1-1.5 GHz) the penetration is quite good for all the fat layer thicknesses considered.

N. R. Amon, I. Mahbub, and P. K. Saha (Amon, Mahbub and Saha, 2017), studied S-parameters and path loss of the tissue model at frequency range 1-5 GHz to estimate reflected power and received power by tissue. Path loss was found to be around 47-65 dB at 3.1-4.5 frequency band, this frequency range allows high speed rate transmission for in-body and on body communication.

V. Mishra, N. Kumar in (Mishra *et al.*, 2016) suggested a new multilayered human head model that having electrical properties (effective dielectric constant) resemble to real human head to compute E-field, absorbed power density (APD), and SAR.

In this study, the parameters affecting propagation characteristics of the electromagnetic field and specific absorption rate (SAR) were analyzed in multi-layered structure of the body shell, thyroid, and testis phantom layers respectively. These parameters included input impedance of the tissue layer (Z_{mn}), complex reflection coefficient between various tissue layer interfaces (K_{mn}, the characters *mn* denote to layer number). Moreover, the distribution of the electrical field (E-field) inside multilayered tissue structure and specific absorption rate (SAR) with corresponding penetration depth (δ) are analyzed in all suggested models. The study is accomplished at three resonant frequencies over UWB frequency span (3 GHz, 6.5GHz, and 10 GHz).

1. HUMAN ORGANS MODELING

The human tissue exposed to strong electromagnetic radiation from wireless sources faces health hazards. This is due to the fact that most wireless communication devices have sufficient power to establish health hazards. The most susceptive human organs to thermal radiation effect and for the frequencies between 150 MHz and 10000 MHz are body-shell, testis, eyes, and thyroid (Velayutham, Sivan Arul Selvan and Unnikrishnan, 2015; Baby, Koshy and Mathew, 2017; Adah *et al.*, 2018).

In this research, the effect of electromagnetic radiation on the body shell, the thyroid glands, and the testis human organs model is studied. These organs were selected specifically because they are the most parts that are close to the power source at the start of the call, where the mobile device is usually in the pocket and during dialing where the mobile is in proximity to the neck, and in both cases, the wireless terminal transmits high power. The human organs structure is quite complex consist of several tissue layers each one has distinct electrical properties. However, the anatomy of the concerning organs with their respective phantom models are shown in Figure 1.

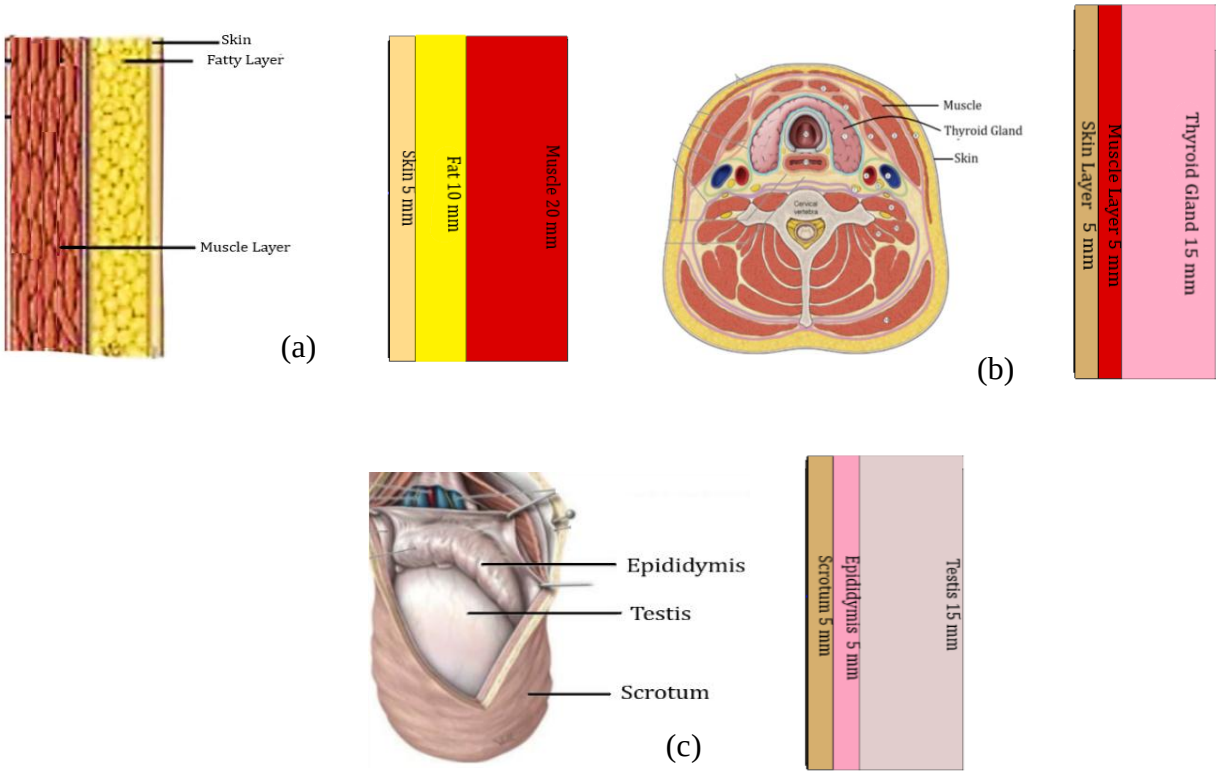


Figure 1. The anatomy of various human organs with their respective phantom models ;(a) body shell, (b) Thyroid Gland. (c) Testis

The human tissue consists of oxygen, hydrogen, nitrogen, and carbon (Klemetsen, 2012), and has frequency-dependent properties. All required electrical properties of human tissue are available in the database of (Hasgall *et al.*, 2015). Table (1) shows some of the electrical properties for the considered tissue and at frequencies of interest.

The body shell, thyroid glands, and testis organ phantom models have been developed with dimensions described in section 4. The models developed to have similar electrical characteristics to that of living organ according to data in the Table 1. However, the multilayered model is adopted in this research to provide a replica of biological organs.

TABLE 1
ELECTRICAL PROPERTIES OF DIFFERENT HUMAN TISSUE AT THREE FREQUENCIES OVER ULTRA WIDEBAND SPAN **(**Hasgall *et al.*, 2015**)**

Tissue type	Conductivity (σ) S/m			Thermal Conductivity W/m/^{0}C	Heat Capacity J/kg/^{0}C	Density (ρ) Kg/m^3
	3GHz	6.5 GHz	10 GHz			
Skin	1.74	4.34	8.01	0.5	3662	1125
Fat	0.344	0.971	1.71	0.24	2973	961
Muscle	2.14	5.82	10.6	0.56	3799	1178
Thyroid	2.44	6.71	12.1	0.53	3609	1050
Epididymis	2.65	6.94	12.4	0.52	3778	1120
Testis	2.65	6.94	12.4	0.52	3778	1120

3.METHOD AND MATERIALS

The essential parameters with their definitions are presented in this section to give good insight about the interaction between microwave signals and biological tissue

3.1. Dielectric Properties

The dielectric properties of the biological tissue play an important role in distribution of the electromagnetic wave inside biological tissue. The complex dielectric constant of a dielectric medium is given by (Kumar and Kumar, 2014) as.

$$\varepsilon_c = \varepsilon' - j\varepsilon'' \qquad (1)$$

Where ε' and ε'' are real and imaginary parts of the complex dielectric constant respectively. The relative complex dielectric constant ε_r is given as;

$$\varepsilon_r = \frac{\varepsilon_c}{\varepsilon_0} = \varepsilon_r' - j\varepsilon_r'' \qquad (2)$$

Where ε_r' and ε_r'' are the real and imaginary parts of the complex relative dielectric constant respectively, and ε_0 is the dielectric constant of free space of value $8.854 \times 10^{-12}\ F/m$.

The imaginary part of the dielectric constant depends on the medium conductivity σ in S/m and angular frequency ($\omega = 2\pi f$) as given by (Kumar and Kumar, 2014) as.

$$\varepsilon'' = \sigma/\omega \quad \text{And} \quad \varepsilon_r'' = \sigma/\omega\varepsilon_0 \qquad (3)$$

Also, the medium loss tangent is given as;

$$\tan\ \delta = \frac{\varepsilon_r''}{\varepsilon_r'} = \frac{\sigma}{\omega\varepsilon_r'\varepsilon_0} \qquad (4)$$

The electromagnetic signals affect the dielectric properties of the tissue through;

1) The electrical conductivity of the tissue, therefore the conduction current with associated energy.

2) The viscosity of the tissue and hence displacement current through the tissue.

Based on database of (Hasgall *et al.*, 2015) and equation 3 the real ε_r' and imaginary ε_r'' parts of the relative complex dielectric constant are calculated for various human tissues, and at three resonant frequencies of interest over UWB span as in Table 2.

TABLE 2
REAL AND IMAGINARY PARTS OF THE COMPLEX DIELECTRIC CONSTANT OF VARIOUS HUMAN TISSUE

Tissue type	Complex relative Permittivity	f_r =3 GHz	f_r =6.5GHz	f_r=10 GHz
Skin	ε_r'	37.5	34.5	31.3
	ε_r''	10.42	11.94	14.49
Fat	ε_r'	10.7	9.67	8.8
	ε_r''	2.05	2.64	3.06
Muscle	ε_r'	52	47.5	42.8
	ε_r''	12.81	15.82	19.29
Thyroid	ε_r'	56.4	50.9	45.1
	ε_r''	14.63	18.51	21.6
Epididymis	ε_r'	56.7	51.1	45.2
	ε_r''	15.88	19.17	22.27
Testis	ε_r'	56.7	51.1	45.2
	ε_r''	15.88	19.17	22.27

3.2.The induced and penetrated Electromagnetic Field into Biological Tissue

The specific absorption rate has been adopted to be a dosimeter quantity of the electromagnetic wave absorbed by the biological tissue and is given by (Stratton, 2007) as;

$$SAR = \frac{\sigma E^2}{\rho} \quad (5)$$

Where, σ is the tissue conductivity (S/m) and ρ is the tissue mass density (kg/m^3).
Some of the incident electromagnetic fields on the tissue penetrate it, and the penetrated field is decreased with tissue's depth exponentially, due to energy dissipation according to the following formula (Greenebaum, 2018);

$$f(z) = F_0 e^{\frac{z}{\delta}} \quad (6)$$

Where *f(z)* is the electromagnetic field as a function of tissue's depth and F_0 is the amplitude of the electromagnetic field signal just at the tissue boundary. The skin depth (or depth of penetration) δ is the depth of the tissue where the field drops to $1/e \approx 0.368$ of its original value of F_0. For the biological medium and over expansive frequency range δ is defined by (Greenebaum, 2018) as:

$$\delta = \frac{1}{\omega \left[\frac{\mu\varepsilon_c}{2} \left(\sqrt{1 + p^2} - 1 \right) \right]^{1/2}} \quad (7)$$

Where μ is the magnetic permeability and for of most biological materials is practically equal to free space permeability $\mu_0 = 4\pi \times 10^{-7}$ H/m, and p is the ratio of conduction current on displacement current and for most biological materials is in the range of $0.1 < p < 10$ (Greenebaum, 2018).

3.3.Input Impedance and Field Distribution in Multilayered Structure of Biological Tissue

The structure of the multi-layered tissue is shown in Figure 2. The structure is composed of four distinct layers arranged as layer 0, 1, 2, and 3. Layer 0 and layer 1 represent the free space and the skin in all proposed models respectively, layer 2 represents Fat, Muscle or Epididymis layers in body-shell, thyroid, or testis model respectively. The last layer (Layer3) represents one of the Muscle, Thyroid, or Testis layers in body-shell, thyroid, or testis model respectively. These models exposed to EM radiation as shown Figure 2.

The reflection and transmission characteristic of multi-layered structure tissue is quite difficult. Multiple reflections can occur between the first and subcutaneous tissue boundaries (Johnson and Guy, 1972). Schwan (Schwan and Li, 1956) adopted a method for describing electromagnetic waves behaviour in multilayer biological tissue. The method defines a unique propagation mode of the electromagnetic waves between layer 0 and layer 3 in biological tissues.

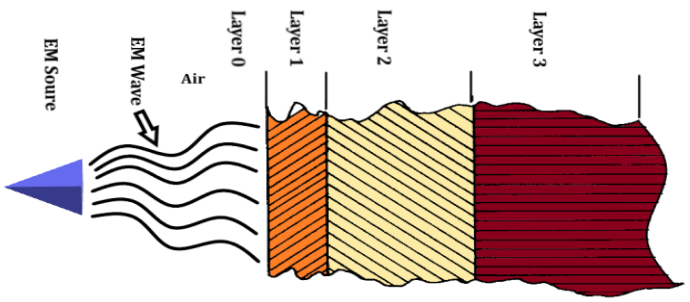


Figure 2. Human Tissue Layers Arrangement

The characteristic wave impedance for each tissue layer is obtained by dividing the wave impedance of air (377 ohm) by the square root of the complex dielectric constant ε_c as in following (Schwan and Li, 1956).

$$Z_l = \frac{377}{\sqrt{\varepsilon_c}} \quad (8)$$

The propagation constant of the radiation $\gamma = \alpha + j\beta$ is obtained by the equation:
Where α is attenuation constant, and β is phase constant.

$$\gamma = j\frac{2\pi}{\lambda}\sqrt{\varepsilon_c} \quad (9)$$

By assigning single digit subscription $_{1,\ 2,\ 3}$ for first, second, third layer respectively, and digit $_0$ for the air, and double subscription ($_{23,\ 12,}$ and $_{01}$) for the layer interface between third-second, second-first, and first-air layers respectively. The following equations are hold good for the tissue layer characteristic impedance, input impedance,

and complex reflection coefficient $K = k\,e^{j\Phi}$ (Schwan and Li, 1956).

$$Z_3 = Z_{23} = \frac{377}{\sqrt{\varepsilon_{3r}}} \quad (10)$$

$$Z_{12} = Z_2 \frac{1+K_{23}e^{-2\gamma_2 d_2}}{1-K_{23}e^{-2\gamma_2 d_2}} \quad (11)$$

$$Z_{01} = Z_1 \frac{1+K_{12}e^{-2\gamma_1 d_1}}{1-K_{12}e^{-2\gamma_1 d_1}} \quad (12)$$

$$K_{23} = \frac{Z_{23}-Z_2}{Z_{23}+Z_2} = \frac{\sqrt{\varepsilon_{2r}}-\sqrt{\varepsilon_{3r}}}{\sqrt{\varepsilon_{2r}}+\sqrt{\varepsilon_{3r}}} \quad (13)$$

$$K_{12} = \frac{Z_{12}-Z_1}{Z_{12}+Z_1} \quad (14)$$

$$K_{01} = \frac{Z_{01}-377}{Z_{01}+377} \quad (15)$$

Where d is the corresponding tissue material thickness.

The tissue interfaces mismatch causes reflection for the incident waves from tissue boundary resulting so-called standing wave, and its strength is given as (Schwan and Li, 1956).

$$E = E_0[e^{-\gamma z} + Ke^{+\gamma z}] \quad (16)$$

Where z is tissue depth coordinate, and E_0 is the peak magnitude of the field at the input tissue layer (boundary) and determined by the boundary conditions.

The standing wave phenomenon becomes significant in each tissue layer if the thickness of the layer is less than the penetration depth for that tissue layer (Greenebaum, 2018).

4. Simulation Setup and Results

The dipole antenna is simulated using CST-2014 software package to be a source for irradiation the incident electromagnetic wave on the human tissue phantom model. The dipole antenna has been resonated for considered frequencies 3GHz, 6.5 GHz, and 10 GHz. The antenna is situated at distance (50) mm away from tissue phantom model with dimension 75×75 mm^2, and thickness 5mm,10mm, and 20 mm, these values are empirically selected for the first, second, and third tissue layer respectively as in Figure 3. The reference impedance of the antenna is considered as a 50 Ω. The input discrete port is defined for an antenna with the input voltage and current equal to 1 volt and 1 ampere respectively. The return loss (*RL*) which is the factor that indicate how well the matching between the transmitter and antenna has taken place. The *RL* is given by (GHAFAR, 2005) as:

$$RL = -20log|K| \;\; dB \quad (17)$$

Where, K is the reflection coefficient. For practical applications a return loss of -10 dB is applicable. The return loss response for the suggested antenna at frequencies of interest is shown in Figure 4.

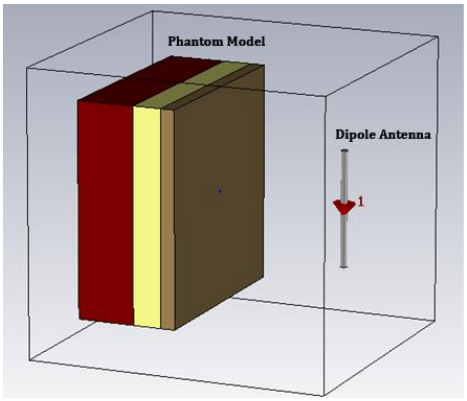


Figure 3 Simulation setup for the antenna with tissue phantom.

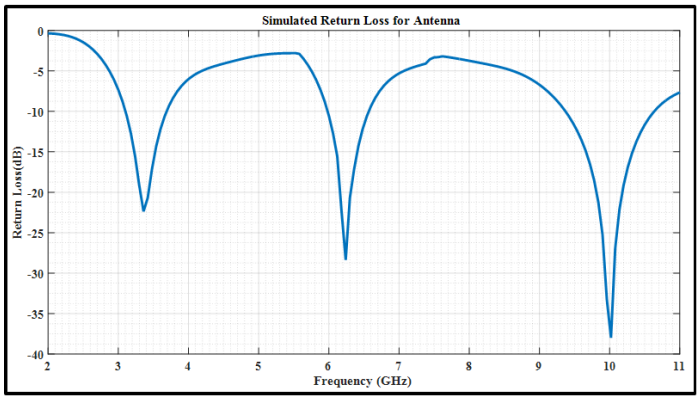


Figure 4 Dipole antenna's response

4.1.Reflection Coefficient (K) of Multilayered Tissue Structure

The incident electromagnetic plane wave with linear polarization on the interface between two media will be partially reflected. The equations (13, 14, and 15) describe the computational analysis of the reflection coefficient in biological multilayered media. If the dielectric properties of the two mediums are comparable or in other words if their characteristic

impedances are approximately equal, most of the incident signal is passed to the second medium, and only a little part of the incident wave will be reflected backed. In contrast, if there is a considerable difference between their dielectric properties or impedances a little signal will infiltrate to second media, and an almost whole signal is reflected.

Based on the database in (Hasgall *et al.*, 2015) for various electrical properties of tissue, the results obtained from the CST-2014 software for ε' and ε'', and the equations (10) to (15), a MATLAB code has been written to calculate input impedances Z_{01}, Z_{12}, and Z_{23}, and complex reflection coefficients K_{01}, K_{12}, and K_{23} between air-skin interface (interface 01) also between subcutaneous tissue layer interfaces (interfaces 12 and 23) of body-shell, thyroid, and testis phantom models and for all frequencies of interest. The results are summarized in Table (3).

Table 3 shows that the value of input impedance between the skin-air boundary and subcutaneous tissue layers boundaries of all models depend on frequency, and accordingly, the reflection coefficients are also frequency-dependent. Moreover, it can be seen from the Table (3) that the magnitude between the input impedance of the air-skin interface (Z_{01}) increases from 60 to 68 ohms, while the input impedance between tissue-tissue boundaries (Z_{12}, Z_{23}) ranges around 50 ohms and is close to each other. For this reason, it can be noted that the magnitude of the reflection coefficient of the air-skin interface (K_{01}) is relatively large (around 0.7) because mismatching with the free space which has a characteristic impedance of 377 ohm, while the magnitude of the reflection coefficient between a tissue-tissue interface (K_{12}, K_{23}) is less than 0.1 because they have comparable dielectric characteristics consequently closer characteristic impedances. Table (3) also demonstrates that there is a little change in phases for both input impedances and reflection coefficient.

TABLE 3: INPUT IMPEDANCE AND COMPLEX REFLECTION COEFFICIENT FOR BODY SHELL, THYROID, AND TESTIS PHANTOM MODELS AT 3GHZ, 6.5GHZ, AND 10GHZ RESONANT FREQUENCIES

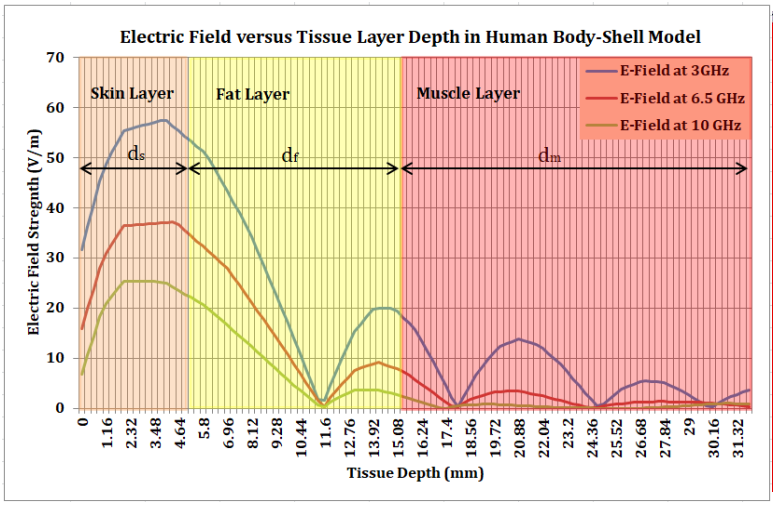
Organ	**Frequency**	**Input impedance**			**Complex Reflection Coefficient**		
Body-shell Layers (Skin, Muscle, thyroid)	3GHz	Impedance	Mag	phase	Reflection coefficient	Mag	phase
		Z_{23}	51.51	-6.92	K_{23}	0.3786	-178.3
		Z_{12}	114.21	-5.42	K_{12}	0.3087	3.43
		Z_{01}	60.42	-7.76	K_{01}	0.7260	-177.45
	6.5 GHz	Z_{23}	53.28	-9.21	K_{23}	0.3819	-178.25
		Z_{12}	119.06	-7.64	K_{12}	0.3127	2.73
		Z_{01}	62.39	-9.54	K_{01}	0.7196	-176.7
	10 GHz	Z_{23}	55.02	-12.13	K_{23}	0.3842	-177.17
		Z_{12}	123.5	-9.58	K_{12}	0.3170	4.02
		Z_{01}	64.19	-12.42	K_{01}	0.7152	-175.68
Thyroid model (Skin, Muscle, Thyroid	3GHz	Z_{23}	49.38	-7.27	K_{23}	0.0213	-171.60
		Z_{12}	51.51	-6.91	K_{12}	0.0800	174.93
		Z_{01}	60.43	-7.72	K_{01}	0.7260	-177.46
	6.5 GHz	Z_{23}	51.22	-9.99	K_{23}	0.0208	-160.85
		Z_{12}	53.28	-9.21	K_{12}	0.0788	177.88
		Z_{01}	62.39	-9.54	K_{01}	0.7196	-176.76
	10 GHz	Z_{23}	53.31	-12.79	K_{23}	0.0168	-159.81
		Z_{12}	55.02	-12.13	K_{12}	0.0770	178.12
		Z_{01}	64.19	-12.42	K_{01}	0.7152	-175.68
Testis							

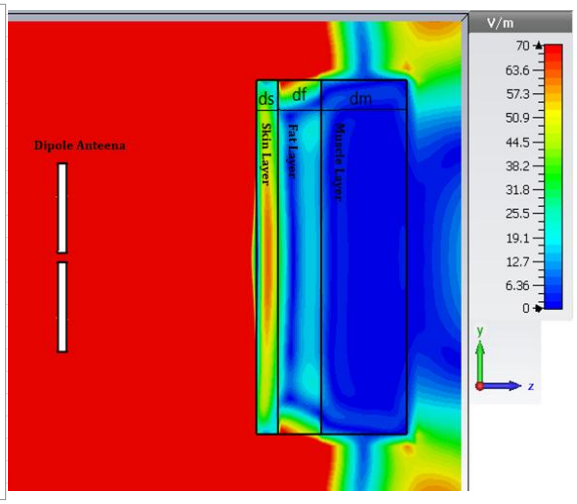
Model (Skin, Epididymis, Testis)	3GHz	Z_{23}	49.13	-7.82	K_{23}	0	0
		Z_{12}	49.13	172.17	K_{12}	0.1033	-179.31
		Z_{01}	60.45	-7.67	K_{01}	0.7259	-177.48
	6.5 GHz	Z_{23}	51.03	-10.28	K_{23}	0	0
		Z_{12}	51.03	-10.28	K_{12}	0.1004	-176.36
		Z_{01}	62.39	-9.54	K_{01}	0.7196	-176.76
	10 GHz	Z_{23}	53.11	-13.11	K_{23}	0	0
		Z_{12}	53.11	-13.11	K_{12}	0.0947	-176.36
		Z_{01}	64.19	-12.42	K_{01}	0.7152	-175.68

4.2Electrical field Distribution in Multilayered Human Tissue Structure

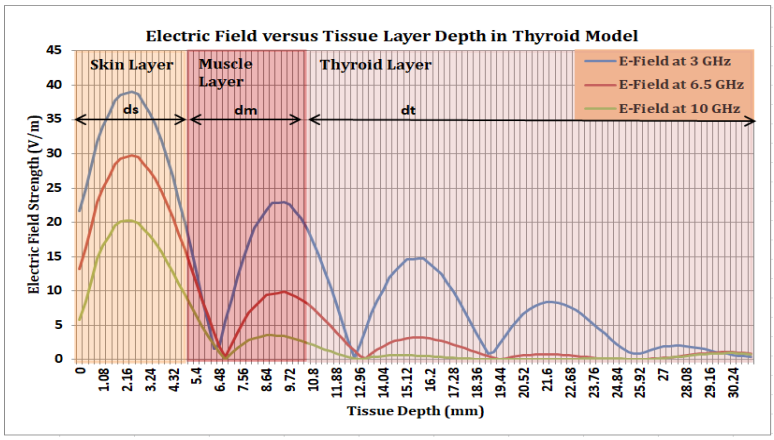
The field analysis in multilayered models for the tissue of the human organ is quite difficult than the single layer. In the presence of multilayer (skin layer and subcutaneous layers) each with unique dielectric properties, multiple reflections can occur between these layers. The reflected wave is combined with an incident wave to constitute a standing-wave phenomenon in each layer. The standing- wave becomes significant in the tissue layer if its thickness is less than its penetration depth (Greenebaum, 2018).

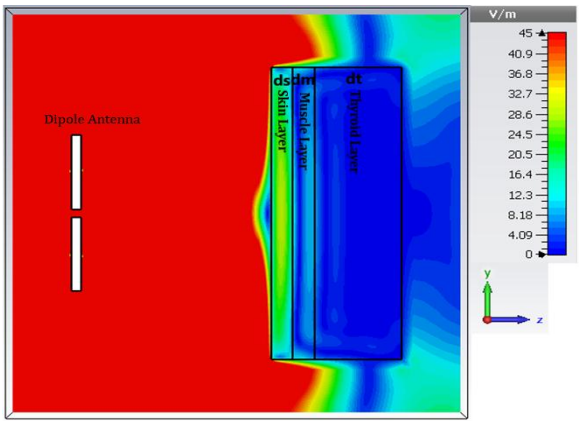
The equation of the electrical field distribution in biological tissue was given by equation (16). Figure 5 show the CST simulated results of the induced electric field distribution along the phantom depth coordinates (*z*) in the multilayered structure of body-shell, thyroid, and testis phantom models respectively.



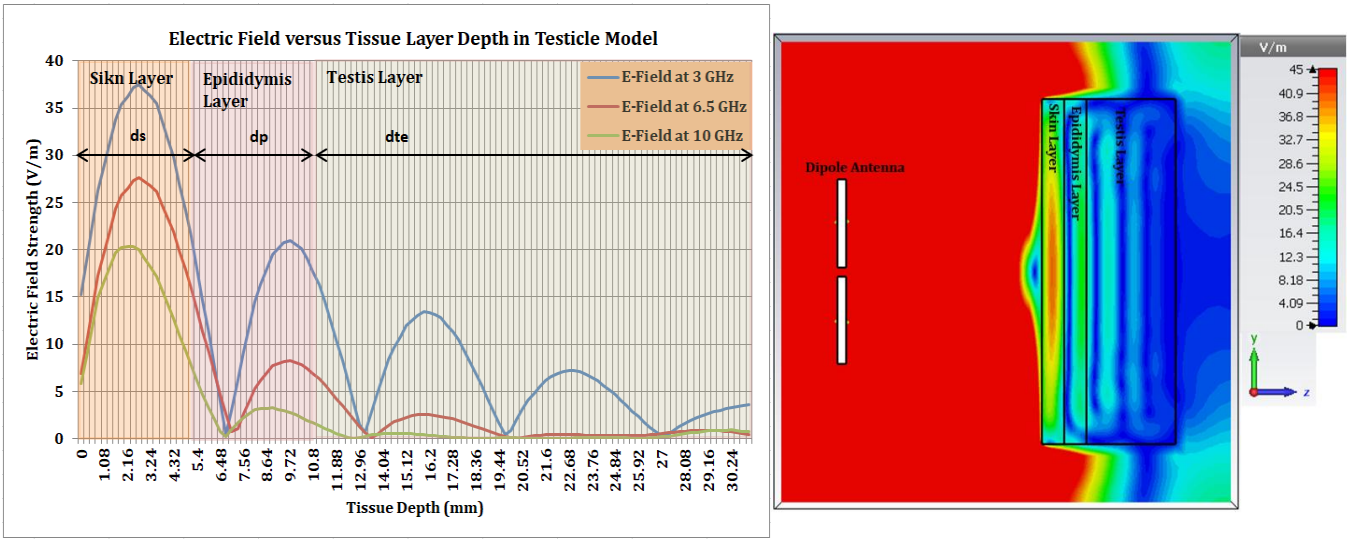


(a)





(b)



(c)

Figure 5 Distribution of the magnitude of the electrical field along tissue depth coordinate (left column), and 2D cross section depiction at 3GHz (right column) for; (a) Body-shell phantom, (b) Thyroid phantom, and (c) Testis phantom.

Figure (5) reveals a set of important points as a follow:

1. The strength of the induced electrical field in the skin layer and all subcutaneous layers depends on the frequency, and for all proposed models. Where the electric field strength (E-Field) at the boundary surface of the air-skin layer, and at the frequencies 3GHz, 6.5GHz, and 10 GHz are 31.1V/m,15.8V/m and 6.8V/m respectively for the body-shell phantom, and 21.6 V/m, 13.1 V/m, and 5.7 V/m respectively for thyroid phantom, also for the testis phantom are equal to 15.2 V/m, 6.8 V/m, and 5.8 V/m respectively.
2. The strength of the electric field decreases as the signal penetrates more distance in tissue layer.
3. The phenomenon of standing-wave more significant in low frequencies than high frequencies (i.e., in the case of 3 GHz higher than 6.5 GHz which is in turn higher than 10 GHz) as stated in (Greenebaum, 2018), where the peak values of the standing wave signal in first layer (skin layer) at 3GHz, 6.5GHz, and 10 GHz frequencies are 57.4 V/m, 37.1 V/m, and 25.2 V/m, respectively, in the body-shell phantom, and 39 V/m, 29.5 V/m, and 20.2 V/m, respectively, in thyroid phantom model, also, in the testis phantom are equal to 37.4 V/m, 27.6 V/m, and 20.4 V/m, respectively.
4. The value of the standing-wave phenomenon in first layer (skin layer) of the phantom models is greater than the second layer (either Fat, Muscle, or Epididymis), and second layer in turn greater than third layer (either Muscle, thyroid, or testis), and this because the thickness of the first layer is less than the second layer which in turn less than the third layer as compared with skin depth.

4.3. Specific Absorption Rate in Phantom Models

The energy of the electromagnetic (EM) waves that strike the human tissue is absorbed by the tissue. The specific absorption rate (SAR) factor measures the rate of absorption of the EM waves in biological tissue as described in equation (5). The absorbed energy undergoes a successive reduction in its magnitude as it launches in the tissue layer as declared in the equation (7).

Figure 6 shows the simulated specific absorption rate in body-shell, thyroid, and testis model versus tissue depth coordinate (z) and for the 3GHz, 6.5GHz, and 10GHz respectively.

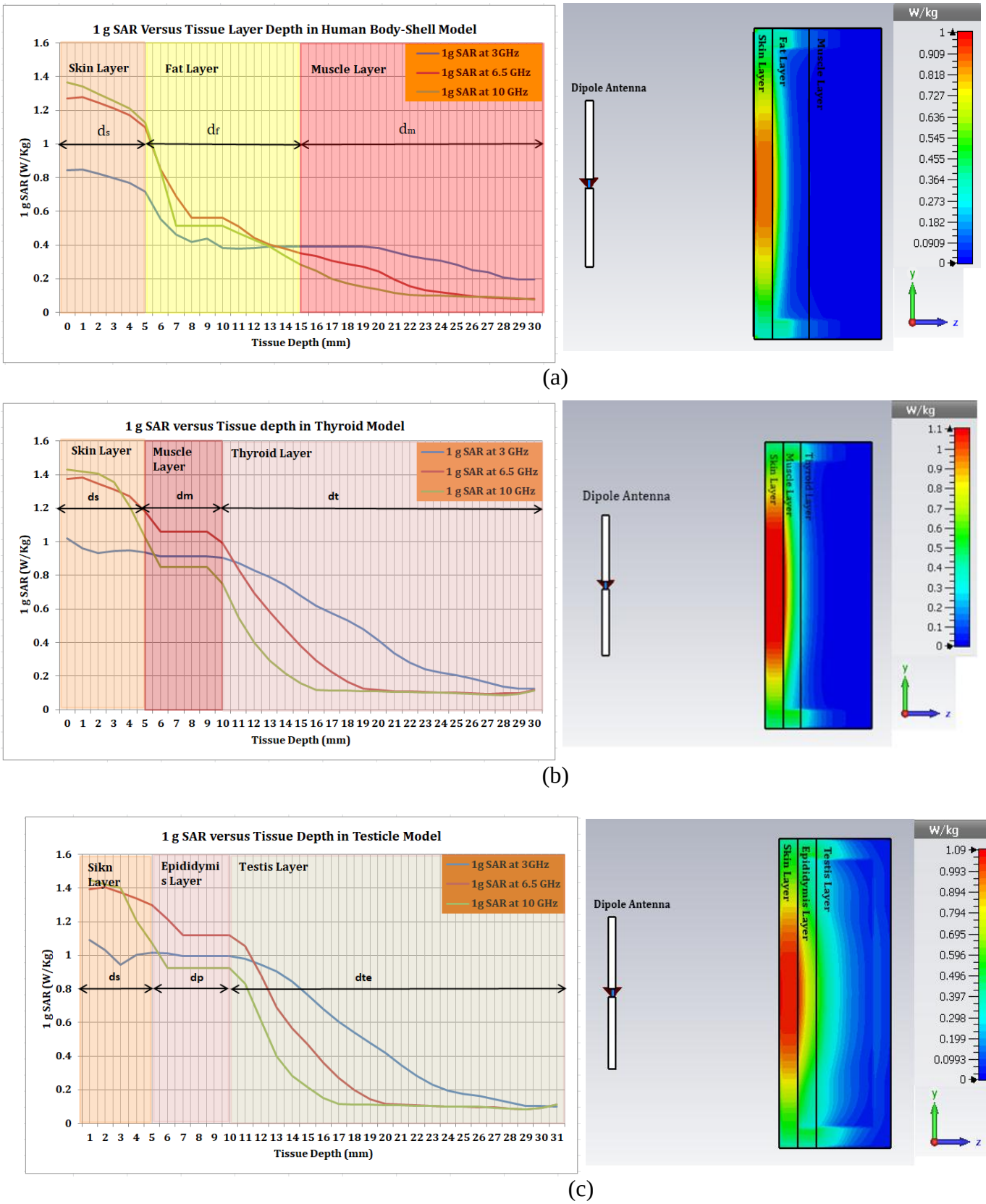


Figure 6: SAR Distribution along tissue depth (left column), and 2D cross section depiction at 3GHz (right column) for; (a) Body-shell phantom, (b) Thyroid phantom, and (c) Testis phantom.

Figure 6 demonstrates decreasing SAR value versus penetration depth. Moreover, the SAR depends on the frequency of the EM signal.

The important point to be noted from Figure (6) is how skin-depth (δ) decreases with increasing frequency for all tissue models, where it is seen at

the distance 0 mm (skin boundary) the SAR value is high for higher frequencies, which is at the frequencies 3 GHz, 6.5 GHz, and 10 GHz equal to 0.84 W/kg, 1.27 W/kg, and 1.36 W/kg respectively for the body shell phantom, and based on 1g measure standard. These values are equal to 1.02 W/kg, 1.37W/kg, and 1.43 W/kg, respectively, for thyroid phantom. Similarly, for the testis phantom these values are somewhat larger than their peers in other phantoms, which are equal to 1.09 W/kg, 1.39W/kg, and 1.45 W/kg respectively. The SAR values are sharply going down after penetrating a little distance in the tissue layer for higher frequencies. This proves the equation (7) that shows the inversely proportional relationship between skin-depth and frequency. Figure 6 also shows that the skin depth (δ) (the depth of tissue at which the SAR value drops to 0.368 of its value at the boundary (i.e., at tissue depth=0mm) as described in section 3.2) at the three resonant frequencies 3GHz, 6.5 GHz, and 10 GHz are 23mm, 12mm, and 7mm respectively for the body-shell phantom. For the thyroid phantom and at the corresponding frequencies are equal to 21mm, 13mm, and 11mm respectively. Regarding the testis phantom, these values are comparable to those in thyroid phantom, which are 19mm, 13mm, and 11mm respectively.

5. CONCLUSIONS

The investigation involves the study of tissue medium characteristics (input impedance *Z* and reflection coefficient *K*), the electrical field (E-Field), and specific absorption rate (SAR) inside multilayered tissue phantom. Several important points are included in this study. Firstly, the input impedance of the first layer boundary (air-skin) is about 60-68 ohms with corresponding reflection coefficient *K* is relatively large (about 0.7). While the input impedance for subcutaneous layers boundary (tissue-tissue) is around 50 ohms with a corresponding reflection coefficient around 0.1. Secondly, the electrical field (E-field) and the SAR absorbed by tissue depend on the incident wave frequency, which there is a direct relationship between them. Thirdly, the penetration depth (δ) is inversely proportioned with the frequency of EM waves. Lastly, the standing wave phenomenon due to tissue layers boundary interface is inversely proportioned with the absorbed wave frequency, where the standing wave becomes more significant when the penetration depth greater than the thickness of the tissue layer. Therefore, the standing wave in the skin layer is greater than in the subcutaneous layers because its thickness is less than the subcutaneous layer thicknesses.

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RESEARCH PAPER

Barriers to the Adoption of Industrialized Building System in Iraqi Construction Industry

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A B S T R A C T:

Iraq construction industry is about to experience an increasing number of new projects. With the economy slowing over the past few years, the need for residential projects and infrastructure still as priorities. Meeting these needs will be challenging, and cash out will be in high range if the construction industry still uses the conventional construction method. The significant features and factors were created based on an extensive literature review of previous research studies in developed and developing countries (68 research papers). Iraq adopted the Industrialized Building System (IBS) approach in the last decades in a few projects, some challenges stand against the continuous adoption of (IBS). Also, many studies record some challenges that prevent the application of IBS in their countries. This study paper discusses the barriers and strategies to adopt IBS in the construction industry of Iraq that are related to the importance of construction development within the context of modern building application. The methodology based on structured hypotheses that employed an online data collection and a total number of (111) questionnaire forms collected from the experts, professionals, and engineers involved in Iraq's construction industry. The questionnaire survey results addressed the barriers, and by using SPSS (statistical package for social science), the researcher correlates the presence of these barriers with hypotheses and demonstrates that the IBS adoption in Iraq suffered a lack of governmental support, money, knowledge, and expertise. Moreover, the related internal productions for successful IBS applications indicated a need to advertise and support the modern construction system by the role of public and private sectors.

KEY WORDS: IBS; Iraq; Construction industry; Barriers; Modern building systems
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ZJPAS (2021), 33(3); 30-42.

1.INTRODUCTION:

Industrialized Building System (IBS) is defined as a construction process that utilizes building components construction equipment. Components are produced on or off-site considering the controlled environment it is transported, placed and assembled into a framework with less additional required work. developing countries, mainly depending on.

There are many identified elements and factors integrated into Industrialised building system (IBS) in the construction industry that include significant macro aspects: 1) Economic attributes that determining the success of adopting IBS in developing countries, mainly depending on funds allocated to develop and solve various construction industrialisation demands that are merged with modern-day urbanisation, the high-cost rate for present-day building construction, and congested commune area. Furthermore, increasing the amount of fiscal multiplier life cycle leading to a securer economy (Shamsuddin, Zakaria and Mohamed, 2013). 2)Social awareness on innovation systems integrated to solve many daily problems such as housing, environmental, economic, and feasible structures, thus having a

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macro scale into developing communities and raising living standards for all population categories. 3)Future planning for a more sustainable and renewable establishment for complex buildings integrating a life span of cost, quality, and durability. Furthermore, strategic planning's continuous process defines a thriving industry in managing all the challenges and constraints occurring in the day-to-day ground (Yunus, Hamid and Noor, 2019).

Lam, Riley, and Tucker (2009) analysed different IBS data outcome and determined that were many common indicators factors related to the outcome of IBS construction adopted in many developing countries, showing success in different abundant deliverables in construction projects represented through these significant indicators: (1) client satisfaction with the product and client satisfaction with the service (Jabar, Ismail and Aziz, 2015). (2) High reliability and quality of IBS designed qualifications (Rahimian et al., 2017) (3) handling and management of many aspects of construction challenges on-site such as risk management, quality assurance. (4) Efficient time deliverables for the designated projects (Nor et al., 2013). (5) Reducing material waste and workforces required on-site, consequently reducing much of operating costs that typically take place in a typical construction project (Pitt et al., 2009). Akram and Longden arranged three primary scopes towards sustainable construction: (1) Efficient management of resources throughout a building's life-cycle(Oleiwi et al., 2017), (2) Cost estimation for the construction phases during a different time sets specifically consisting of operation, maintenance, improvement and deconstruction(Faghirinejadfard et al., 2015), and (3) Economic profitability for local Small and Mid-size Enterprises (SMEs) due to the mere fact of consistent demands of resources for the construction of IBS components. Nevertheless, Kamar and Mahdiyar researched further and concluded that financial revenues and reconcilable achievement in securing high-quality building specification were the two most influential drivers that will drive demand and supply for a sustainability socioeconomic deliverables for stakeholders (Shamsuddin et al., 2018). Not to mention, research on welfare facilities requirements in Iraq (Hatem Z.M., Hamid A.R.A, Abba, N., 2019), concluded that IBS is considered one of the most innovative and effective systems in building welfare facilities on construction sites. These involve significant and large scale construction projects in many developing countries (Abba, N., Hamid, A.R.A., Hatem, Z. M., 2019). One of the main obstacles to research into construction industrialization was defining boundaries and establishing a clear description. The construction industry in Iraq still not yet developed as well using new methods such as IBS often interchangeable with another term, and their exact definitions depend in no small extent on the user's experience and understanding.

To discuss the barriers that may influence Iraq's construction industry, a review of collected previous studies conducted to list all the findings and critical barriers frequency. Besides, the respondents' responsiveness on the questions of the survey in a professional community (consultants, contractors, engineers, etc.) and their impact correlation in the construction industry of Iraq. Furthermore, the research papers were divided into six groups to easily categorise and identify the barriers to adopt IBS using a coding method to highlight the practical parts in the research papers, mainly featured in the literature review part. The identified factors were established and considered as a benchmark to refer to in this research Table 1.

Table (1) Frequency of Critical Common Barriers from Previous Studies

No	Barriers to adopting IBS	G1 (Ali *et al*., 2018)(Ibrahim *et al*., 2020)	G2 (Mohd Amin *et al*., 2017)(Rashid *et al*., 2019)	G3 (Akmam Syed Zakaria *et al*., 2018)(Al-Taie, Al-Ansari and Knutsson, 2014)	G4 (Jabar, Ismail and Aziz, 2015)(Mezher, 2019)	G5 (Hadi, Muhamad and Othman, 2017)	G6 (Nduka *et al*., 2019)(Abed, 2018)	Freq. (N)
1.	Cost	√	√		√	√	√	5
2.	Awareness	√		√	√	√		4
3.	Knowledge	√	√	√	√	√	√	6
4.	Attitude	√		√				2
5.	Project delivery	√		√	√			3
6.	Skilled workers	√			√	√	√	4
7.	Design	√			√		√	3
8.	Conventional builds to IBS		√		√	√		3
9.	Enforcement of IBS requirement		√	√			√	3
10.	Cash flow problems		√					1
11.	Industrial objective		√		√			2
12.	Economic condition			√			√	2
13.	Stakeholder participation			√	√		√	3
14.	Sustainability feature			√				1
15.	Risk management						√	1

1. METHODOLOGY

2.1. Previous Case Studies of IBS Implementations:

The sample of this research consisted of 38 case studies that have been selected from reliable resources such as journals (Springer, Elsevier, Taylor & Francis, etc.) and official reports from public and private development sectors. The time baseline was from 2015 to 2020. Data collection methods include semi-structured pilot study, statistical analysis, and extensive literature review. The analysis was to convert literature review content text and empirical data into a reliable indicator by establishing a pragmatic benchmark coefficient for dependent and independent variables. As proposed by (Mogey, 1999), observing the descriptive category of analyses, he concluded that several methods could be adopted in analysis techniques in a way it can be used to analyses the statistics and data to develop some results from the study of the results.

They were using a skewness and kurtosis equations (1,2) for analysis for the data available. The reason for the usage of skewness and kurtosis is to elaborate and clarify to the extent which variable's distribution is symmetrical. The general test is that if the number is greater than 1, the distribution is reliable. Likewise, a kurtosis of less than 1 indicates that it is not efficient. Besides, the technique developed to study the data in this analysis is by using computer software, which is NVivo 12, a qualitative data analysis (QDA) mainly for coding and Statistical Package for Social Science (SPSS) analysis the empirical data. All trials of the analysis showed some resemblance to the need for cost and funding collaboration, project efficient, research knowledge into IBS, manufacturing and risk management, the need experienced professionals,

and adopting a modern building system. The total common indicators for previous research and case studies on IBS were 15 barriers, shared by the title, effect, results, and construction development.

Finally, to correlate all the final data for the standards, Cronbach's Alpha, Kendall's, and Spearman's Equation (3,4,5) were modelled in accordance to determining the coefficient to be used in SPSS software to make decisive results there is a statically indeterminate link between the ratios of indicators. The analysed coefficient is assumed to be equal, or more than 0.7 based on previous errors and trials conducted to establish a reliable benchmark to referee (Abba, N., Hamid, A.R.A., Hatem, Z. M., 2019). The final results were constructed to test the hypothesis on the data obtained for Iraqi respondents and establish a link for the missing aspects needed by the construction industry in Iraq. The summary of the case studies depicted in the following Table 2 and 3:

N = number of items.

$\bar{c}$ = Average covariance between item-pairs.

$\bar{v}$ = Average variance.

S= Standard deviation

$\underline{X}_i$=Initial variance

g2= covariance between Xi and X

X =Mean

G=Range [−1, 1]

C= 5-point scale

d =Ranks individuals

m=Correlation coefficient

G1=Equation(1):Cronbach's equation inputted in SPSS

G2=Equation(2):Spearman's equation inputted in SPSS

α=Equation (3): Alpha coefficient equation

W=Equation (4): Kendall's equation

P=Equation (5): rho coefficient equation

$$G1 = \frac{n}{(n-1)(n-2)} \sum_{i=1}^{n} \left(\frac{xi - \bar{x}}{S}\right)^3 \quad \text{Equation (1)}$$

$$G2 = \frac{n-1}{(n-2)(n-3)}[(n+1)g2 + 6] \quad \text{Equation (2)}$$

$$\alpha = \frac{N.\bar{c}}{\bar{v} + (N-1).\bar{c}} \quad \text{Equation (3)}$$

$$W = \frac{12\,S}{m^2(n^3-n)} \quad \text{Equation (4)}$$

$$P = 1 - \frac{6\sum d_i^2}{n(n^2-1)} \quad \text{Equation (5)}$$

Table (2) Analysis of research papers

No.	Indicators for IBS	Impact scale 2.0	Effect on other elements	Impact summary	Hypotheses classification	Test method
1.	Cost	1.42	1.83	3.25	**Hypotheses 2**	**Skewness and kurtosis interpretation**
2.	Awareness	0.78	1.20	1.98		
3.	Knowledge	1.79	3.64	5.43		
4.	Attitude	0.96	0.00	0.95		
5.	Project Delivery	2.08	6.55	8.63		
6.	Skilled Workers	0.92	-0.26	0.66	**Hypotheses 1**	**Pearson correlation**
7.	Design	1.41	1.68	3.09		
8.	Conventional building To IBS	1.71	2.53	4.24	**Hypotheses 0**	**Descriptive analysis**
9.	Enforcement of IBS	1.02	-0.15	1.16		
10.	Cash Flow Problems	2.62	6.82	9.44		
11.	Industrial Objective	1.61	2.01	3.61		
12.	Economic Condition	1.24	1.04	2.27	**Hypotheses 3**	**Cronbach's alpha coefficient**
13.	Stakeholder role	1.42	1.83	3.25		
14.	Sustainability attributes	0.78	1.20	1.98		
15.	Risk Management	1.79	3.64	5.43		

Table (3) Analysis of final results for the papers

Cronbach's Alpha	Kendall's tau_b	Spearman's rho	N of Items (Research papers)
0.701	0.879	0.930	68

2.2. Hypotheses Methodology

Hypotheses are used to link between testable documents and research questionnaires. Therefore, hypotheses create a correlation to interconnects the gap from the general question that was set to investigate the problems that occur are daily. Furthermore, to generate an effective assumption, relevant explanations of a problem are ascribed by the hypothesised methodology by rationalising the overall connection between variables and constant outcomes(Sawyer, 1991).

To simplify the questionnaires and construction industry's outcome results, an empirical hypothesis is used to link between qualitative and quantitative results(Barker, 1961). This type of research methodology organises the research to a more qualified and accurate. It establishes the dynamic variations that can develop and reduce miscellaneous data; thus, the standard of control in such research is adequate even with different variables. The hypotheses are divided into four stages that are observation, testing, evaluation, and linking(Granato, Inglehart and Leblang, 1996). The hypothesis is divided into four sections to clarify the research objectives; the hypothesis uses dependent variables that are structured for changes in independent variable data from the designed questionnaires:

1. Hypotheses $_0$: Lack of awareness of modern technology building systems and the abundance of traditional building
2. Hypotheses $_1$: Absence of professional's experts to manufacture and execute IBS components in Iraq
3. Hypotheses $_2$: High capital and operational cost that prevent the implementation of IBS construction in Iraq

4. Hypotheses $_{3:}$ Unstable construction market throughout the rates of supply and demand for IBS construction

2.3. Questionnaire Survey

A profound review of previous studies to evaluate the most common barriers and discuss it. The questionnaire survey is also conducted to decide the level of awareness among construction industry players towards the adoption of IBS and the issues that stand against the complete application in the Iraqi construction industry. A systematic google form of quantitative and qualitative approach distributed in an experienced stakeholder forum to answer the questions for this research. In the meantime, the qualitative approach covered a list of questions that aim to encourage the respondents to answer these questions based on their opinion and site experiences. Some of the questions need the respondents to answer subjectively. The questionnaire survey continued to collect responses from the respondents for two months. A total number of one hundred and ten (110) questionnaire responses were received as the study sample. Statistical package of social science (SPSS) used to analyse the data obtained. This selection is based on previous study that calculated the minimum sample size in respect to the Iraqi construction industry (Hatem Z.M., Hamid A.R.A, Abba, N., 2018). The instrument of this survey included three parts. Information related to the respondents' background, years of experience (Rashid et al, 2019), type of construction. Correlation equation used to find the extent correlation of these barriers in the construction industry.

3. RESULTS AND DISCUSSION

The survey result discussed, as shown in the next section. This questionnaire created by google Forms and distributed among professionals and experts who deal with construction activities in their daily work. A total number of 110 respondents send their feedbacks relating to some personal background information and experiences.

3.1. Background of the Respondents

In this section part (a), the data analysed for the background of the respondents. In the figure1 site, engineers represent a considerable percentage of 38% compared to the other slices that may direct the questionnaire results to rely on the respondents' experience to specify the barriers. The consultants and the contractors also form 11% and 13% respectively.

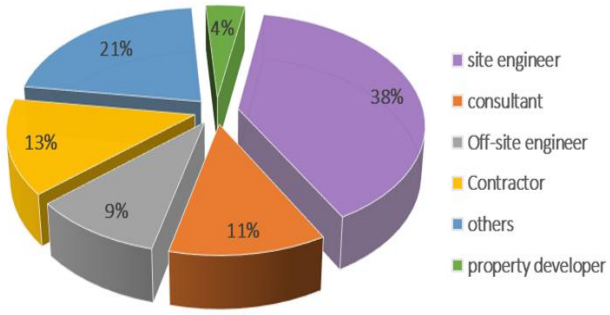

Figure 1: Job/Position of the respondents

In figure 2, the respondents' level of education shows that bachelor and master engineers are the highest numbers recording 43%, 36%, and in collaboration with the previous figure means to emphasise experience and practical knowledge to address the problem and barriers of this research.

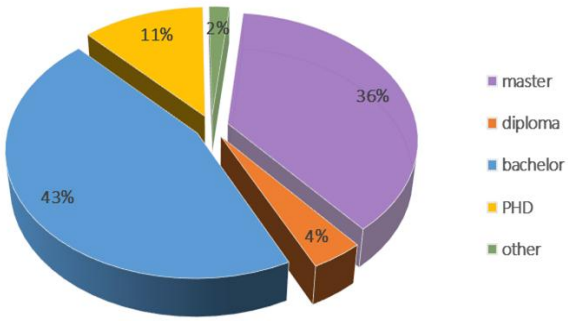

Figure 2: Level of education

A different percentage of 58% is shown in figure 3 concerning the respondents involved in the construction industry from 1 to 5 years and an approximately equal percentage to the other options of this questionnaire 11%, 13%, and 15%.

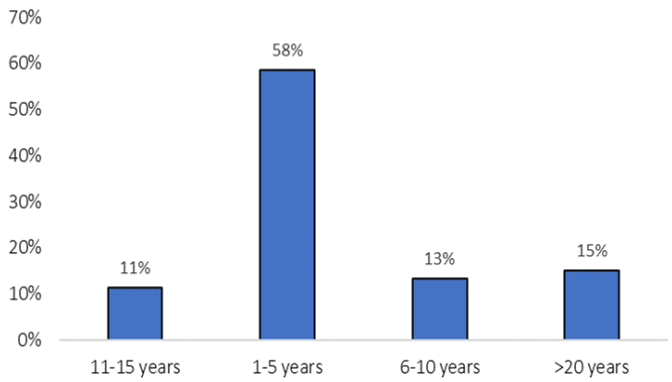

Figure 3: Years of experience in the construction industry

Figure 4 shows that the residential units record 40% concerning the type of buildings developed by the respondents. It is crucial to consider the opinion of this sector to address the barriers and facilitate the application of IBS in a wide range. The respondents of the Commercial and industrial projects show an equal percentage of 17% of the questionnaire's total population.

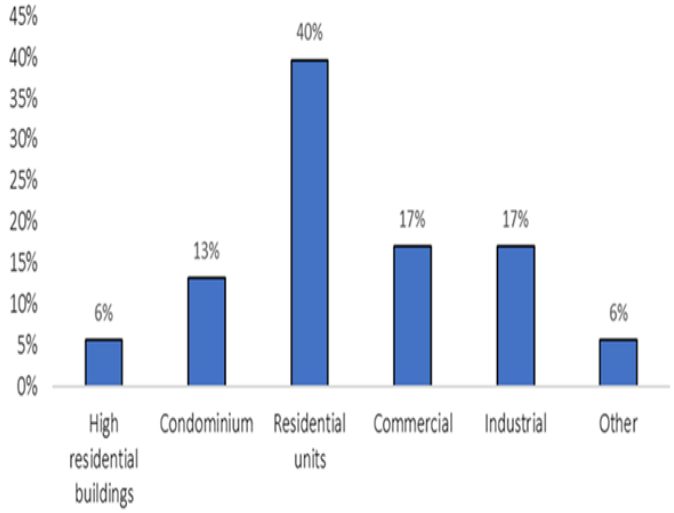

Figure 4: Building types developed by the respondents

3.2. Results of The Questionnaire Barriers

This part (b) is to analyse the objectives listed and problems, in so an analysis is performed to test the hypothesis theory using both quantitative and converting it to empirical statics Table 4 using several systems to effectively evaluate and prove the critical components of the hypothesis constructed to fit Iraq's existing conditions Figure 5. The system used to correlate and correct any misapprehension responses of the surveys. A correlated series is established to test the indicators' validity as illustrated in Table 4 using Pearson correlation in SPSS (Abba, N., Hamid, A.R.A., Hatem, Z. M., 2019). The parameters used in the analysis is correlated to the values obtained from the barriers values created from the systematic analysis of previous studies, survey response values, and structured coefficient of mathematical limits of accuracy for testing the listed hypothesis.

Table (4) Indicators correlation using the parameters

Indicators	Signified indicators	Pearson Correlation	Test
	Series 1	Series 2	
Cash Flow Problems	.990	0.98	✓
Convert from Conventional To IBS	.978	0.86	✓
Design	.973	0.47	✗
Economic Condition	.950	0.34	✗
Cost	.910	0.36	✗
Skilled Workers	.921	0.07	✓
Transportation	.830	0.76	✓
Attitude	.773	0.97	✓
Limit Enforcement of IBS Requirement	.721	0.69	✗
Awareness	.697	0.49	✗
Industrial Objective	.629	0.77	✓
Stakeholder Participation	.539	0.202	✗
Sustainability Feature	-.239	0.06	✗

Furthermore, its noticed that in accordance to the previous case studies of IBS in developing construction industry, the similarity is led to several spot factors that consider an impactable drivers for advancing and changing construction methods of building. As such these are resembled from Table 4 and Figure 4 of mainly funding issues that diverges into other causes and developing changers as regarded in previous experiments and notes of already developed nation. Noticed in the trials and errors conducted in the scope of management and world leading industries as reviewed in the selected cases of this research.

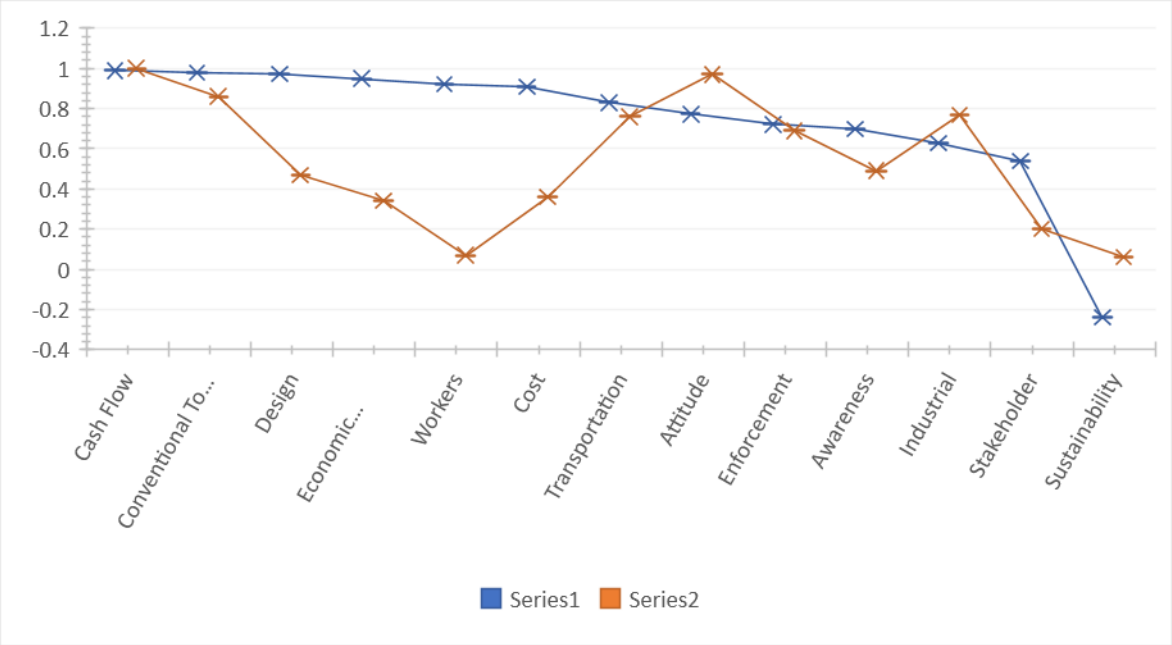

Figure 5: IBS barriers related to construction industry management

For part (c), the data received questionnaires responses are analysed in SPSS. A descriptive statistic is directed for distribution numbers and repeated variables in phases of uniformity and potential deviations. Many researchers recommend that the correlation system is applicable to examine the relationship between combinations of variables. To properly analyse the relationship, a correlation concept is used mainly know by Pearson's product-moment correlation (r). Pearson's correlation coefficients are typically adopted for usually disbursed data which shows the linear relationship between two or more variables. Pearson's correlation coefficients (r) equation (1,2) adopts a statistics range of interval from -1 to +1 Table 4. To establish the strength of variance of two variables compared, coefficient of resolution is calculated and measured on Pearson's range of -1 to +1, using a cross-product feature in SPSS to create a gauge of compelling correlation represented in positive figures closest to +1 that represents the most significant barriers to implement IBS system in Iraq, this is illustrated and defined in the vertical axis in Figure 5. Followed by forming IBS standards to be referred to in an empirical method and analysis, economic conditions and cash flow were the most significant barriers preventing IBS building in Iraq with an average coefficient of (0.9) Figure 4 and is clearly resembled in the chart in Figure (5). Series1 represents the values obtained from the analyse of previous studies that are focused in IBS application. Series 2 represents the final values analysed from the respondents in context to IBS barriers.

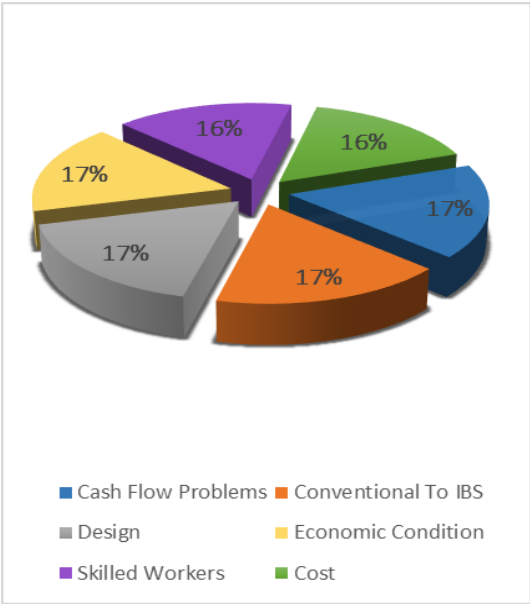

Figure 6: Financial Role

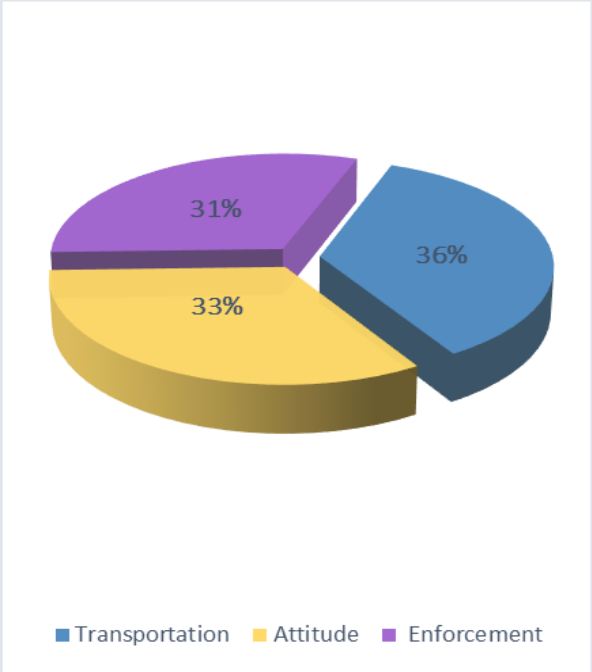

Figure 7: Public and Private Sectors Role

There are not many reliable records about IBS ideas in Iraq, and the government has to act in this regard. Modern media play the prominent role in making people familiar with the advantages of IBS and educating them about advanced building systems around the world; however, with a low coefficient on this subject (0.69), there is a need to increase the understanding on this system. The existence of the defined standards for sustainability in public and private agencies is significate low with a (-0.239) coefficient. In other suitable buildings are yet to be introduced in Iraq construction industries. The primary stakeholder and government officials responsible have a significant impact on applying and encouraging IBS in Iraq, as indicated in the analysis with an average coefficient (0.7). Material availability and manufacturing IBS components are available to be invested and explore the potential of manufacturing. As for the transportation and lifting equipment needed, Iraq has various heavy moving vehicles and lifting cranes, as shown in results averaged (0.8) Figure 5 and the survey's results indicate that in figure 8.

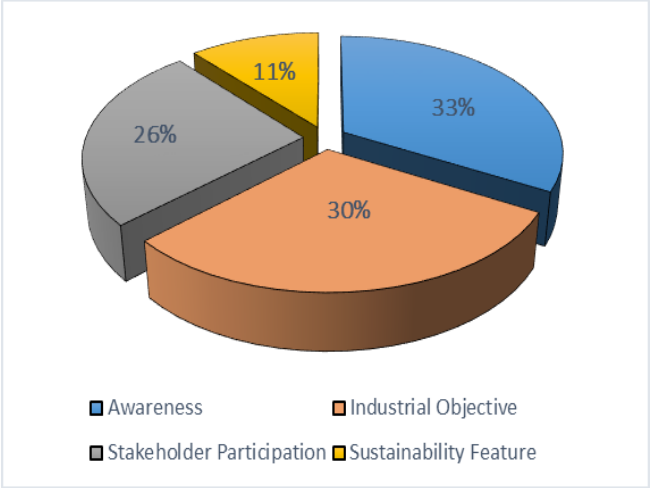

Figure 8: Significant Stakeholders' Impact

In conclusion, Iraq is in a deficient situation in terms of the degree of models defined for the use of industrial building systems (IBS), furthermore. The result also pointed out that more than half of respondents admit the application and potential benefits of using modern building and construction systems in Iraq. According to the results, the most popular project type needed is residential and housing (refer to Table 2: Analysis of research papers), Considering IBS is an efficient solution to this continuously occurring issue Figure 3. The final results for the obtained analysis are shown in Figure (4), highlighting and illustrating the most impact factors considered as barriers for the IBS system in Iraq. Both figure (3,4) results with previous studies conducted in developing countries, where an installation of new building and construction knowledge is lacked, thus a survey concurs with the barriers previewed in the literature review in a mathematical and descriptive interpretation. Also the leading results that are illustrated in the figure above point to many neglected or in other word in need of more studies for leading to improvement of construction industry in accordance to world standards in application and consideration.

For the analysis part of hypotheses (3), Cronbach's alpha is used to evaluate the attributes of internal consistency factors and how closely linked a set of components are as a systematic classification, which is in the case of research IBS barriers. As a consequence of the analysis, the following was revealed, knowledge and training importance of the IBS concept in this research is represented as a fair reach awareness on the basic needs to achieve and fulfil IBS potential without

neglecting ethics and local traditions. From the retrieved analysis data, it reveals, there is knowledge of the essential potential aspect of aesthetic IBS details in Table 5. Furthermore, there are three factors interested between the hypotheses parts, mainly found in the critical prospect of education and coordination between the construction industry and universities (higher education research and development institutes).

However, the rest aspects related to construction industry contribution mainly by public sectors showed little importance, which indicates that there must be communication between the primary development sector to establish IBS Figure 9.

Table (5) Cronbach test related to indicators

Indicator	Cronbach's Alpha	Test hypotheses
Local workers impact	.625	✕
Design integrated with IBS	.630	✕
Aesthetic features	.769	✓
Qualifications in Design and Production	.597	✕
High-level educational institution into IBS	.766	✓
Effective supervision techniques	.622	✕
IBS effect on construction	.658	✕
Extensive coordination before construction operations	.725	✓

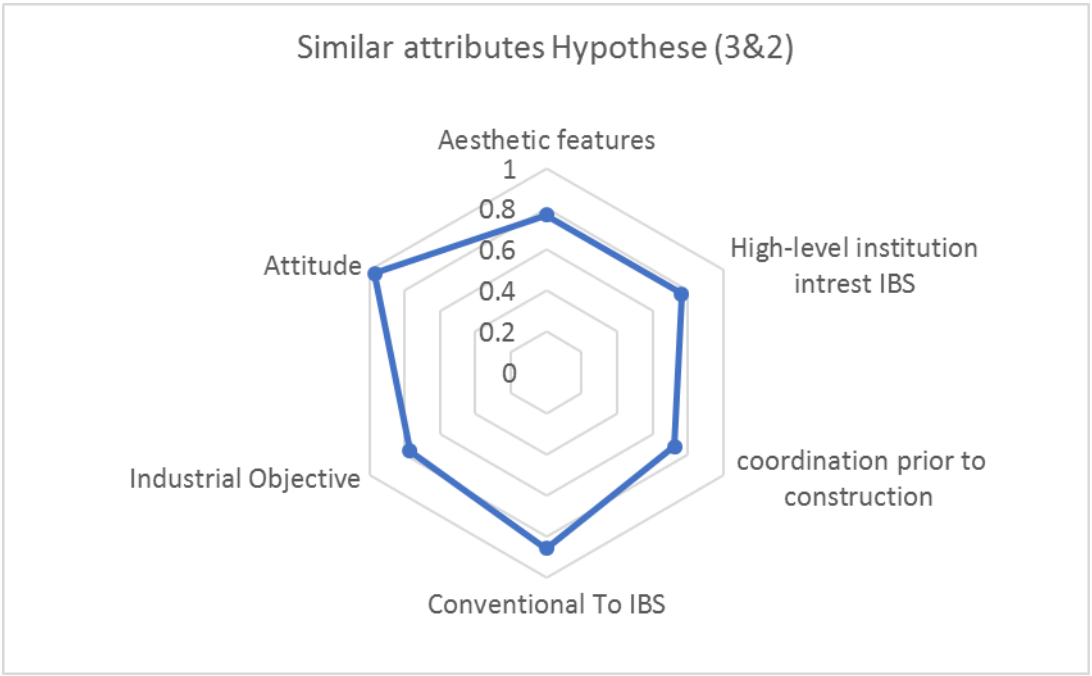

Figure 9: Similar attributes of hypotheses (3&2) based on Cronbach's Alpha coefficient

4. RECOMMMENDATION

To overcome the barriers towards implementing IBS that addressed in the literature review and the questionnaire survey, the respondents assure that the application of IBS should be taken immediately. In the respondents' opinion, the first step should be taken by the government to encourage institutes, public sector to adopt the idea of converting from conventional to IBS in a wide range, support, and plan strategies in the building and constructions sector. The government should lead the initiatives and enhance the application of this system in the private sector. IBS added as a mandatory option at least for the need for residences and housing units. Furthermore, the government should support fabrication units, produce skilled workers, and

create opportunities for Iraqi labourers and workforces (Mahdi and Mawlood, 2020; Mahdi, Wali and Yuosif, 2020). To increase the application of IBS, the government should support some companies well known in this field of constructions to invest in future projects and change the ideas of the people towards the implementation of the IBS system giving high-quality specifications and futuristic design. Seminars, advertisements, and documents need to promoted in a wide range to increase people's awareness of the positive side of using the IBS system. To stimulate the demand and supply of the IBS system, the government should think about tax reduction, help to increase the Number of IBS manufacturers by giving flexible financial plans for investors.

5. CONCLUSIONS

From the analysis and interpretation, it can be decided that the most significant elements towards the barriers of IBS in Iraq's construction are the financial aspect, awareness into modern construction system, and most importantly the crucial role of the public and private sector to adopt IBS in their construction development and future needs for continuous demand for improvement in building supply. The particular peculiarity of IBS will benefit and promotes more investment in construction. With the addition, the adoption of this alternative construction method, there must be full awareness of the capacity of IBS in enhancing construction features such as cash, time, quality, safety, and sustainability environment. An incorporated appraisal process and effective collaboration are required between major influencers (Designers, Planner, Engineers, Governments officials, and Researchers) related to IBS application in Iraq on the key attributes. Also, from the results obtained and in testing the basic hypothesis a concurrence can be generated with the appliance of previous cases conducted in the aspect of IBS, in explaining and linking the results the methodology was affluent in drawing into summary for interpretation (Ali, M. M. *et al.* 2018). As for the null hypothesis, the descriptive results show that the theory for the barriers of IBS is in accordance with previous statement of scholars that highlighted the main factors that contribute to application of modern construction systems in developing countries (Mohd Amin, M. A. *et al.* 2017).

As this concurs with previous studies conducted in other construction related aspects that followed a similar pattern for determine and concluding the importance of construction development context within the daily practice and guidance of Iraq's current perspectives(Hatem, Hamid and Abba, 2019; Hatem, 2020).

Acknowledgements

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RESEARCH PAPER

Cumulative effects of low X-ray doses on some liver function and proteins of diagnostic technicians working in conventional X-rays

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A B S T R A C T:
Today X-rays are widely used for diagnostic in medicine using different technics such as computed tomography (CT), fluoroscopy and conventional X-ray. During diagnostic procedures the technicians might expose to low X-ray doses especially those who omit radiation protection tools. The aim of the study was to assess liver function and proteins due to the cumulative effects of low X-ray doses on diagnostic technicians through measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins, albumin, globulin, serum ferritin (s.ferritin), malondialdehyde (MDA) and glutathione (GSH). Twenty four male diagnostic technicians at Kirkuk hospitals participated, they classified into two groups depending on their working experiences, each group with 12 technicians. Twelve male healthy controls participated to show any alteration of the parameters. The results showed that ALT, AST and s.ferritin increased significantly ($p<0.05$) for the first group compared to the control while high significant ($p<0.001$) increasing was recorded for the second group. Total proteins decreased significantly ($p<0.05$) in the first group and high significant ($p<0.001$) decreasing in the second group compared with the control group. High significant ($p<0.001$) decreasing for albumin was recorded in both groups while globulin decreased insignificantly ($p>0.05$) in the first group and significant ($p<0.05$) decreases were recorded for the second group. For MDA and GSH high significant ($p<0.001$) increasing and decreasing respectively, recorded for both groups. The study revealed that low doses of X-ray can change some liver functions and proteins and the number of working years has discernible effects on the cumulative doses in diagnostic technicians.

KEY WORDS: Cumulative dose, X-ray, Proteins, Diagnostic, liver functions
DOI: http://dx.doi.org/10.21271/ZJPAS.33.3.5
ZJPAS (2021), 33(3);43-50.

1. INTRODUCTION:

Ionizing radiation was used for diagnosis and therapy in medicine broadly especially after the discovery of X-ray by Roentgen in 1895. The medical use of radiation widespread to a lot of countries in the world during the twentieth century, and is becoming more familiar (Agency 2001). Today X-rays are widely used for diagnostic in medicine using different technics such as computed tomography (CT), fluoroscopy and conventional X-ray (Brenner and Hall 2007), ((Hall and Brenner 2008),(De Santis, Cesari et al. 2007)). There are risks associated with X-ray imaging as in many aspects in medicine. The most common effects are the induction of cancer after a long period of years ((Hall and Brenner 2008), (De Santis, Cesari et al. 2007), (Brenner 2010)). X-ray classified as carcinogenesis by World Health organization (Roobottom, Mitchell et al. 2010). Different factors such as types, energy and doses of the radiation, age, individual health and volume of body exposure to have the main rule on the biological effects of ionizing radiation. Different types of effects may be produced after exposure to the radiation such as deterministic and stochastic effects. Deterministic effects result from acute exposure while stochastic effects resulting from chronic exposure. Deterministic effects occur due to cell death or delay in cell division, if large numbers of cell die to affect the function of the

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tissues or organ. In stochastic effects the cells are not dying but alter in some way causing delay effects such as carcinogenesis and hereditary effect ((Bohm, Hendry et al. 2010), (Podgorsak 2005)).

During diagnostic procedures the technicians might expose to low X-ray doses especially those who omit radiation protection tools. Exposures to low doses of the radiation for a long time are the main risk factors in X-ray imaging ((Cengiz, Gurkaynak et al. 2003), (Hashim, Karim et al. 2016), (Protasova, Maksimov et al. 2001)). In the first half of the past century many of serious health problems such as increasing of skin cancer and leukemia along with enhanced cancer and all cause death rates have been reported in radiologic technologists and radiologists (Dublin and Spiegelman 1948). It was demonstrated that increasing breast cancer risk in US cohort radiologic technologists is due to occupational radiation exposure in low to intermediate dose range (Preston, Kitahara et al. 2016), however the above cases happened before the recommendations of protection implemented by international organizations.

It has been proved that there is a relationship between ionizing radiation and malignant diseases ((Pierce and Preston 2000), (Ochs 2011)) including various liver diseases presenting with tender hepatomegaly, hyperbilirubinaemia and ascites ((Lawrence, Robertson et al. 1995),(Helmy 2006)) . It has been proved that ionizing radiation can induce hepatic dysfunction or liver cancer in patients without liver diseases who undergo radiotherapy ((Walsh, Grosche et al. 2015), (Ozasa, Shimizu et al. 2012),(Pan, Kavanagh et al. 2010),(Zielinski, Garner et al. 2009)). Liver can be damaged by high doses of ionizing radiation but the effects of chronic exposure of the radiation on liver damage are not clear (Sun, Mao et al. 2018). (Bakshi, Azimzadeh et al. 2015) showed that liver inflammation in male mice increase after exposed to single dose ranging from 0.02 to 1.0 Gy. Although, the liver was once believed to be relatively radio-resistant, hepatic morphologic and functional alterations have been noticed after radiation therapy ((Tai, Erickson et al. 2009), (Gore and Levine 2010)).

MDA is the last product of peroxidation; major aldehyde product that is mutagenic in cells and could be used to assess cell damages ((Marnett 2002), (Dalle-Donne, Rossi et al. 2006)).GSH is a common antioxidant in human body and participated in human defense system against oxidative damage. It has been reported that GSH level can be reduced by oxidative stress (Sener, Kabasakal et al. 2006).

Several studies have been done on the cumulative effects of low X-ray doses on the different parameters in diagnostic technicians. (YASMIN, GARIMA et al. 2009) showed that X-rays can inhibit the immune activity and induce damage to kidneys and liver in radiologic technicians. (Taqi, Faraj et al. 2018) reported that some hematological parameters in diagnostic technicians can be significantly changed after chronic exposures to X-ray and work duration has discernible effects on the some parameters. (Nureddin and Alatta 2016) concluded that long-term of work to low X-ray dose may cause a low degree of severity of diseases which is expressed in term of hematological changes.

Biological impacts of low doses of the radiation still are not clear, so more researches are required. However, studies on the effects of low doses on proteins and liver function are limited, the aim of the current study was to assess the cumulative effects of low X-ray doses in diagnostic technicians through measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), total proteins, albumin, globulin, serum ferritin (s.ferritin), malondialdehyde (MDA) and glutathione (GSH).

2. Materials and Methods

Twenty four male diagnostic technicians at Kirkuk hospitals participated in the study, depending on their working experiences they classified into two groups, each group with 12 technicians, the mean work duration of the first group was 6.2 years ranging from (3-9) years with the average age (45.3±6.6) years and the second group was 17.2 years ranging from (10-25) years with the average age (33.8±8.9) years. Twelve male healthy controls was with the average age (40.5± 8.5) years selected from outside of the hospitals, they are non-smokers, non-taking any medications, they did not have any diseases before and during the study.

The aim of the study was explained to all participates and their participation was optional. Questionaries' data was filled out then the amount of 5 ml of blood was taken from the veins of each participate. All the technicians worked on the conventional X-ray machine, five of the

technicians were smokers and two of them had hypertensions.

AST, ALT, total protein and albumin were measured by a technique according to the instructions of manufacturer company kit (Randox), while s.ferritin was measured by using ELISA technique. Globulin was determined using the following equation:

Globulin= total proteins - albumin

MDA was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer (Rao, Soufir et al. 1989). GSH level estimated by mixing 2.3 ml buffer with 0.2ml of the sample and then added 0.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture was analyzed by spectrophotometer (Moron, Depierre et al. 1979).

During performing this study at the hospitals, the technicians did not use radiation protection tools for themselves and patients.

The t-test was used for a statistical difference between the means of the measurements in diagnostic technicians and control groups. P values < 0.05 were considered as statistically significant and P values < 0.001 considered as high significant.

3. Results

The cumulative effects of X-ray doses on ALT, AST and total proteins in the diagnostic technicians are shown in Table 1 and Fig.1, ALT, AST and s.ferritin increased significantly ($p<0.05$) for the first groups compare to the control while high significant ($p<0.001$) increasing was recorded for the second group. The mean values of ALT were (18±3.83) U/L, (25.16±9.81) U/L, (60.83±14.89) U/L for control, first group (work duration 3-9 years) and second group (work duration 10-25 years) of the technicians respectively. The values of AST for control, first and second groups were (16.25±2.86) U/L, (25.33±9.61) U/L, (59.83±19.48) respectively, and for s.ferritin the mean values were (44.43±3.89) ng/L, (51.55±7.39) ng/L, (64.73±8.48) ng/L for the same order of the groups.

The results for total proteins, albumins and globulins are presented in Table 2. Total proteins decreased significantly ($p<0.05$) in the first group and high significant ($p<0.001$) decreasing was recorded in the second group compared with the control group. The values for control, first and second groups were (7.30±0.29) mg/dl, (6.65±0.37) mg/dl, (5.85±0.60) mg/dl respectively. High significant ($p<0.001$) decreasing for albumin was recorded in the both groups of the technicians while globulin decreased insignificantly ($p>0.05$) in the first group and significant ($p<0.05$) decreasing was recorded for the second group. The mean values of albumin were (3.03±0.33) mg/dl, (2.68±0.28) mg/dl for first and second groups of the technicians and the value for control was (3.59±0.23) mg/dl. Globulin measurements were (3.70±0.37) mg/dl for control group and (3.63±0.31)mg/dl, (3.10±0.42) for first and second groups of the technicians respectively. The results also are shown as a chart in Fig.2.

For MDA and GSH, high significant ($p<0.001$) increasing and decreasing respectively, recorded for both groups compared with the control, the data are shown in the Table 3 and Fig.3. The mean values for MDA were (1.24±0.07) mol/l, (1.52±0.10) mol/l, (1.95±0.19) mol/l and for GSH were (0.44±0.01) mol/l, (0.41±0.02), (0.37±0.03) mol/l for control, first and second groups respectively.

The important results significant difference ($p<0.01$) between first group (work duration 3-9 years) and second group (work duration 10-25) were observed for nearly all measurements which is the main purpose of the study as shown in the tables.

Table 1; AST, ALT and s. ferritin measurements for both groups of the technicians and control

Groups / Parameters	Control Mean ± SD	Diagnostic technicians Work duration (3-9)years Mean ± SD	P-Value	Diagnostic technicians Work duration (10-25) years Mean ± SD	P-Value
AST (U/L)	18±3.83	25.16±9.81*	<0.05	60.83±14.89**	<0.001
ALT (U/L)	16.25±2.86	25.33±9.61*	<0.01	59.83±19.48**	<0.001
S.ferritin	44.43±3.89	51.55±7.39*	<0.05	64.73±8.48**	<0.001

Table 2; total proteins, albumins and globulins measurements for all groups

Groups / Parameters	Control Mean ± SD	Diagnostic technicians Work duration (3-9)years Mean ± SD	P-Value	Diagnostic technicians Work duration (10-25) years Mean ± SD	P-Value
Total proteins (mg/dl)	7.30±0.29	6.65±0.37*	<0.01	5.85±0.60**	<0.001
Albumin (mg/dl)	3.59±0.23	3.03±0.33**	<0.001	2.68±0.28**	<0.001
Globulin (mg/dl)	3.70±0.37	3.63±0.31	>0.05	3.10±0.42*	<0.05

Table 3; Measurements of MDA and GSH for both groups of the diagnostic technicians and control

Groups / Parameters	Control Mean ± SD	Diagnostic technicians Work duration (3-9)years Mean ± SD	P-Value	Diagnostic technicians Work duration (10-25) years Mean ± SD	P-Value
MDA (mol/l)	1.24±0.07	1.52±0.10**	<0.001	1.95±0.19**	<0.001
GSH (mol/l)	0.44±0.01	0.41±0.02**	<0.001	0.37±0.03**	<0.001

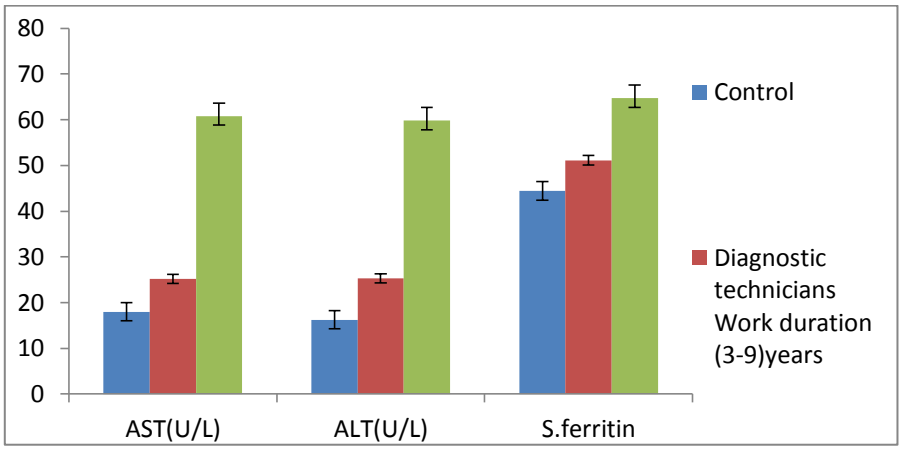

Figure1; Chart of AST, ALT and s. ferritin for the two groups of the technicians and control

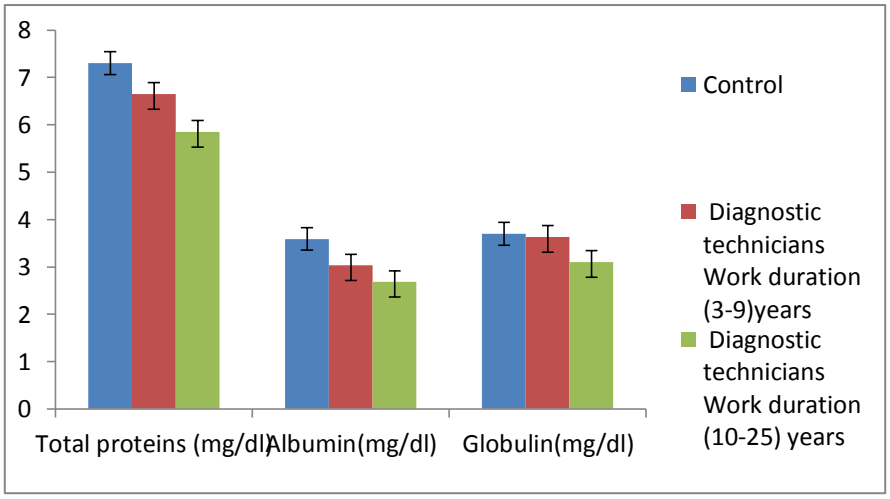

Figure 2; Chart for values of total proteins, albumin and globulin for the two groups of the technicians and control group

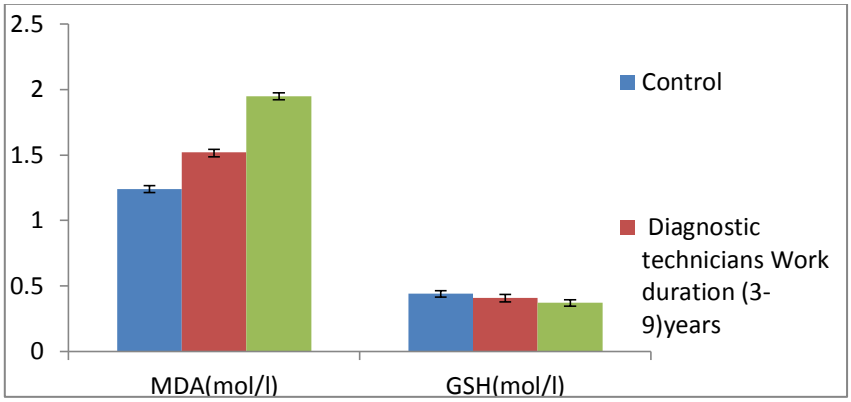

Figure 3; Chart of MDA and GSH measurements for the two groups diagnostic technicians and control group

4. Discussion

Chronic exposure to ionizing radiation may results various health effects (Meo 2004).Diagnostic technicians probably expose to low X-ray doses at their workplace especially those who omit radiation protection tools as was observed at the hospitals during the study. However the technicians during individual procedure exposed to low dose of X-ray but occupational risks arise from stochastic effects. Serious, long-term and possibly fatal adverse health consequences may arise from daily exposure procedures over a long time. It has been known biological effects of ionizing radiation increases with increasing time of exposure.

This study was done for two different duration of the exposure. However studies on the effects of low dose on the proteins and liver function are limited. In this study changing of ALT, AST, s. ferritin, total proteins, albumins, globulins, MDA and GSH were observed between control group and the technicians who exposed for two different duration time depending on their working experiences.

In the current study ALT and AST increases with increasing duration time of exposure. It has been shown that liver is considered a radiation sensitive organ ((Rösler, Christiansen et al. 2015), (Stryker 2007)) and chronic exposure to radiation is a risk factor liver injury (Sun, Mao et al. 2018). (Bakshi, Azimzadeh et al. 2015) suggested that liver function and the development of liver disease in animals may affect after exposure to low dose radiation. (Nwokocha, Nwokocha et al. 2012) showed that the values of ALT and AST elevated significantly with increasing radiations after total body irradiations of rats to 1.27 Gy/minute as cumulative doses.

In this study s. ferritine also increases in the two groups compared with the control. The increasing may be due to oxidative stress and enzymes activity affected by the radiation. It has been reported that the change in ferritin subunits induced due to increasing in hepatic and the direct effect of different inflammatory mediators such as acute-phase cytokines ((Naz, Moriconi et al. 2013), (Torti, Kwak et al. 1988)).It has been shown that many diseases are related with iron overload or iron deficiency. S. ferritin has been described as a risk factor for veno-occlusive disease (Morado, Ojeda et al. 1999). (Feng, Chen et al. 2015) recorded that there is a significant association between s. ferritin and colorectal cancer. It has been shown that determination of ferritin is a suitable method for ascertaining the iron metabolism situation which provides a representative measure of body's iron reserves (Antonini and Albertini 1984).

Decreasing of total proteins, albumins and globulin were observed in the present study with increasing duration time of exposure. (Ditzel 1962) showed that after 1500 rad and 1000 rad of total-body X-irradiation of golden hamster the albumin fraction decreased, and the αl-, α2-, and β-globulin fractions increased. (Goranson, McCULLOCH et al. 1960) observed exposure of the animals to supra-lethal total-body irradiation produced changes in a number of the serum components. The most striking change is the increase in the α-globulin component (Fα2). The results of current study disagree with the above two studies may be due to the difference in the absorbed dose and the duration of exposure. (Nwokocha, Nwokocha et al. 2012) also shown that serum proteins increased significantly with increasing radiation and serum levels of albumins significantly ($p < 0.05$) increased at the beginning of radiation exposures but reduced when cumulative dose exposure increased which agree with the results of the present study.

Oxidative stress induced by radiation may increase MDA which being interested for scientific research and intracellular concentration of GSH can be used as indicator of oxidative stress (Mohamed, Mohammad et al. 2014)**.** In the current study MDA increased significantly while GSH decreased significantly in the groups of the technicians compared with the control group. Decreasing of GSH may be due to low dose of radiation which can induce oxidative stress. (Meydan, Gursel et al. 2011) suggested that ROS cause negative effect on the antioxidant defense by depleting intracellular concentration of GSH. (Deger, Dede et al. 2003) showed that after irradiation of mice to 550 rad X-ray the level of MDA increased while GSH decreased and (Zhao, Wang et al. 2012) recorded that MDA increased and GSH decreased in liver tissues of mice after irradiation to 5Gy of Co-60, the results of these two studies agree with the results of the current study however they used high doses compared with the doses of the current study. Oxidant damage to the mitochondria and myocyte membranes that could promote cell death due to membrane damage termed as radiation induced apoptosis may be due to high MDA level (De Freitas, Boligon et al. 2013). (Qian, Cao et al. 2010) showed increasing of MDA in cardiac tissue of mice irradiated by gamma rays.

5. Conclusions: The present study provides estimation of accumulative effect of low X-ray doses on proteins and some liver functions and it is a basis for more studies on the effects of low radiation doses and accumulative effects on liver functions, proteins and other system. The study revealed that low X-ray doses can change some liver function and proteins and the number of working years has discernible effects on the cumulative doses in diagnostic technicians. The author recommended that more studies on the other effects of low doses of the radiations on human health are necessary to be done. Training and courses was recommended for the diagnostic technicians to improve their knowledge and protect themselves through using radiation protection tools during diagnosing.

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Conflicts of interest: the author declares that there is no conflict of interest.

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RESEARCH PAPER

Estimating the Storativity by Recovery data in Khabat area

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A B S T R A C T:

The study presented a method for estimating aquifer storage coefficient form recovery test data meanwhile, it is hard to determine especially for single well test cases. The method involves an equation based on the principle of Jacob's modified Theis equation by measuring residual drawdown data versus recovery time in the production well. The main objectives of this study is to determine the reliability of this equation in the selected area and to evaluate and compare the results with the pumping test results as well. The method is starting by estimating the Aquifer parameters (Transmissivity and Storativity) which involves plotting residual drawdown as a function of time, later substituting the obtained test values in an appropriate transient groundwater flow equations based on the aquifer characteristics and types to find the parameter values. Finally, the study concluded that the proposed equation is reliable for determine the Storativity of the aquifer for the single well test, and also can be used as a check for the parameters that obtained from pumping test results and thus the method is verified the objective of the study.

KEY WORDS: Residual drawdown; Aquifer Parameters; Storativity; Transmissivity Diffusion.
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1.INTRODUCTION :

Generally, one of the most important subject in developing countries is determining the aquifer parameters. due to its importance in water resources management for the selected area. it is also has a crucial role in groundwater modeling for any basin. The current study tried to find the reliable method for estimating hydrogeological properties of the aquifer form the conducted pumping test and the recovery test as well. Furthermore, during of recovery period when the pump is turned off the drawdowns which starts to raise up to its original place are taken as it is considered in pumping tests.

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drawdowns are is most cases considered more reliable than the pumping drawdowns when it is compared with the results obtained from the aquifer parameters. The time when the pump is shut-off is called as the recovery time.

On the other hand, conducting pumping tests include difficulties in keeping the pumping rate constant, taking account of drawdown in the pumping well due to well loss and turbulent flow in the vicinity of the testing well. However, the Recovery test data are free from these limitations.

Based on the literature, (Theis, 1935) recovery test method is mostly used to determine the Transmissivity from the recovery test data; but it cannot yield an estimate of the aquifer Storage Coefficient such as explained in (Todd, 1980). This can be seen is those cases when the last pumping drawdown is not considered a part of the recovery data. Later, Both (Chenaf and Chapuis,

2002) and (Samani and Pasandi, 2003) used the Cooper-Jacob approximation of the Theis solution and gave straight-line plots in semi log graphs for calculating T and S from recovery data. While their work represented progress in applying the recovery data for estimating of the pumping test, and also their methods were limited due to using of the Cooper-Jacob approximation, which requires that both pumping and injection phases have proceeded for long enough that both arguments (u1 and u2 are no more than 0.01), to ensure sufficiently small truncation errors. While it is often reasonable to expect (u1< 0.01) to hold during the recovery phase, the requirement of (u2 < 0.01) would exclude the use of many early-time recovery data.

In addition, a method selected to estimate T and S from only residual drawdowns observed at a single observation well was probably first proposed by (Case et al., 1974). Also, (Bardsley et al., 1985) provided a least- squares method to estimate T and S using only residual drawdowns. These methods cannot identify a changed storage coefficient during recovery; also, it can be ascertained using (Singh, 2003) method that the data used by the authors for the application of these methods do not pertain to confined aquifers, hence are inconsistent with the methods used. Estimating the accurate values of aquifer parameters required conducting pumping test on the production well and measuring drawdown in the monitoring well .but it needed high cost of economy, so in order to decrease cost of other well ,it is regarded to used many methods for that test which is known as single well test. There are a lot of attempt to estimate Storativity for single well test ,such as effective well bore storage (Choi, Byoung –Soo, 2007), Recovery test(P. N. Ballukraya and K.K. Sharma,1991), and (G.P. kruseman, and .de Ridder.1973) and (Mawlood D. K 2019a) which studied the sustainability for erbil basin. slug test,..ect. In this case due to the absence of the observation well the application of diffusion equation is selected for ground water flow in the vicinity of the well . Based on (Mustafa J. S , 2017) pumping test is conducted by pumping water from the well itself at a constant rate and measuring the drawdown in the well as a function of time. The test data are used to understand how water is stored and moves through the aquifer towards the well .The analysis of test result is used to determine hydraulic properties of the aquifer .

2 MATERIALS AND METHODS

2.1. Study Area Location

Khabat District is located on the west of Erbil province in KRG, Iraq, near Greater zab river and The location is at the distance of about (37 km) from Erbil city, that is located on longitude of (36°16′20.48″), and latitude of (43°40′ 23.99″) with the elevation varies between (200 to 400 m.a.s.l). see Fig No.1:

Figure 1: Satellite image of the study area location.

2.2. Methodology of the study

The study described a method for finding the Storativity of the aquifer by finding the Transmissivity from recovery test data, then by finding the radial distance from observation well through diffusion equations such as described in detail. the importance of the method is that the Storativity cannot be found without observation well distance, however in this study this radial distance presented to find out by diffusion equation then the results of the Storativity will be within the standard range of the aquifer parameters. Recovery test starts when the pump is turned off during pumping test and at the same time the water levels in the pumped wells will start to rise to its initial test level. The measuring water level in this period is known as residual drawdown, which can be represented by the distance from the static water level (SWL) to level during rising and the time also called time of the recovery test. It is verified that the residual drawdown test due to recovery is a good practice to calculate the accurate Transmissivity of the aquifer and to be used as check for the results. [

Kruseman and Di Ridder, 1990]. Moreover, the obtained residual drawdown data are more reliable than pumping test data, due to the constant rate of the recovery test, whereas keeping constant pumping rate at the time of pumping test is rather difficult.

The method is based on the principle of the superposition. Then the provided data on the plots substitutes in the derived equations and the parameters can be determined.

2.3 Application of Recovery test methods

All Usually the Recovery test starts when the pump is shut off during the well testing, the water level starts to rise and the system approach to the equilibrium, then water goes back to its original level and dynamic water levels (DWL) can be measured with respect to time. the study shows the appropriate methods for analyzing the recovery test of the conducted well by substituting the well test and recovery data in the proposed equation then finding the hydraulic properties of the Aquifer, the analyzing method based on the Theis well functions equation after modification by Jacob for long period of time the values of u near to zero so the long part of the Theis equation can be neglected and the equation can be used on this bases. If the following assumptions considered into account then Jacob modified Theis (1935) equation can be applied for the estimation of the recovery data, as follow;

For this principle, Theis (1935) showed that the residual drawdown s´ can be given as:

$$s'=\frac{Q}{4\pi T}[W(u)-W(u')] \quad (1)$$

$$W(u) = [-0.5772 - \ln u + u - \frac{u^2}{2.2!} + \frac{u^3}{3.3!} + \cdots] \quad (2) \qquad u = \frac{r^2 S}{4Tt}$$

$$W(u') = [-0.5772 - \ln u' + u' - \frac{u'^2}{2.2!} + \frac{u'^3}{3.3!} + \cdots] \quad (3) \qquad u' = \frac{r^2 S'}{4Tt'}$$

where t and t´ are the times since pumping started and stopped, respectively, s´ is residual drawdown, Q is the constant well discharge, r is radial distance from pumped well, S and S´ are the storage coefficients of pumping and recovery period, and T is Transmissivity.

It was noted by Cooper and Jacob (1946) and Jacob (1950) that for large values of time with u or u´ small, that is, less than 0.01, the series terms for W(u) or W(u´) are negligible after second term. Then, the drawdown can be calculated as follows, with an assumption that

It should be noted that the Storativity in case of pumping test = Storativity during recovery test (S=S´):

$$s'=\frac{Q}{4\pi T}[\ln\frac{2.25Tt}{r^2S}-\ln\frac{2.25Tt'}{r^2S'}] \quad (4)$$

simplified the equation to obtain the following equation:

$$s'=\frac{Q}{4\pi T}\ln(\frac{t}{t'}) \quad (5)$$

or:

$$s'=\frac{2.3\,Q}{4\pi T}\log(\frac{t}{t'}) \quad (6)$$

$$\text{Slope}=\Delta s'=\frac{2.3\,Q}{4\pi T} \quad (7)$$

where

W(u) : well function of pumping test (unitless)
W(u´): well function of recovery test (unitless)
Q : Constant pumping Rate (L3/T)
s : Drawdown during pumping (L)
s' : Residual drawdown during recovery (L)
Δs': Change
t : Time during pumping test (T)
t' :Time during recovery test (T)
r : Radial distance from pumping well to point of measuring drawdown (L)
T : Transmissivity (L^2/T)
S : Storativity of pumping test (unit less)
S' : Storativity of recovery test (unit less)

However, (Jacob,1946) derived a method based on (Theis,1935) equation for large values of time (t) and small value of u and for time versus drawdown after considering Jacob's assumptions (C. W. Fetter, 1952). It can be used:

$$s=\frac{2.3Q}{4\pi T}\log_{10}\frac{2.25Tt_o}{r^2S} \quad (8)$$

To calculate the value of Transmissivity through Jacob's equation:

$$T=\frac{2.3Q}{4\pi\Delta s} \quad (9)$$

Based on the (Kruseman and de Rider 1990), the above equation is used to estimate Transmissivity, the slope of the line (Δs) on the semi logarithmic plot are taken by measuring the difference between two drawdown per one log cycle ,and the intercept of the straight line at zero drawdown determines the initial pumping test

time(t_o), then using it to estimate storage coefficient (S) in the equation below:

$$S = \frac{2.25Tt_o}{r^2} \qquad (10)$$

After finding the Transmissivity and Storativity from pumping test data, then the results substitute in presented diffusion equation to find reliable S' value due to Recovery data. see test data in Table 1:

Table (2) : Pumping and Recovery test data

Time (min)	depth to water (m)	s (m)	t (min)	t' (min)	t/t'	s' (m)
0.00	63.00	0.00	140.50	0.50	281.00	8.88
0.50	68.40	5.40	141.00	1.00	141.00	6.68
1.00	70.40	7.40	141.50	1.50	94.33	4.15
1.50	71.30	8.30	142.00	2.00	71.00	2.38
2.00	72.10	9.10	143.00	3.00	47.67	1.79
3.00	73.10	10.10	144.00	4.00	36.00	1.49
4.00	73.60	10.60	145.00	5.00	29.00	1.29
5.00	74.10	11.10	146.00	6.00	24.33	1.09
6.00	74.50	11.50	147.00	7.00	21.00	1.00
7.00	74.75	11.75	148.00	8.00	18.50	0.90
8.00	74.80	11.80	149.00	9.00	16.56	0.80
9.00	74.90	11.90	150.00	10.00	15.00	0.70
10.00	75.00	12.00	155.00	15.00	10.33	0.40
15.00	75.00	12.00	160.00	20.00	8.00	0.20
20.00	75.00	12.00	165.00	25.00	6.60	0.10
30.00	75.00	12.00	170.00	30.00	5.67	
40.00	75.00	12.00	180.00	40.00	4.50	
50.00	75.00	12.00	190.00	50.00	3.80	
60.00	75.00	12.00	200.00	60.00	3.33	
80.00	75.00	12.00				
100.00	75.00	12.00				
120.00	75.00	12.00				

2.4 Recovery Test Data Analysis

The study is in the Khabat area which is belong to Erbil province and the test conducted on the single well in Khabat area and the well having the coordinate of Easting 381349m, Northing 4014240 m and also the elevation of (334 m) above sea level. Moreover the depth of the well is 265m and the casing diameter 0.22 m with the effective well radius of 0.16 m, also the static and dynamic water level were 82.7m, 106.2 m respectively the drawdown inside the pumped well was 23.6m the well discharge is 1600.57 m3/day and the aquifer saturated thickness of the production well is 182.3m, based on (Ismail, O. Srwa, 2020).

By plotting data on semi-logarithmic paper the value of Transmissivity T can be obtained from the recovery data using Jacob modified Theis equation which is the graphical solution, and values directly substituted in the equation to obtain the Storativity of the aquifer as shown in Fig.2:

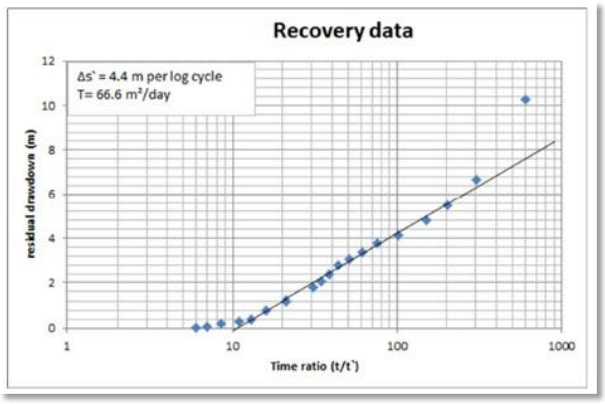

Figure 2: Recovery test data analysis.

Although the following steps should be taken into account during the calculation in order to get the values of both Transmissivity and Storativity as the following calculations:

$$T = \frac{2.3*1600.57}{4*\pi(4.4)} = 66.61 m^2/d$$

Storativity obtained from the test is (S=0.16) based on the study (Mawlood D. K 2019c) and finding Storativity based on radius of influence by diffusivity equation:

$$\eta \frac{1}{l^2} = \frac{1}{\mathcal{T}} \qquad (11)$$

$$l^2 = 4\eta\, T \qquad (12)$$

$$L = 2\sqrt{\eta Tt} \qquad (13)$$

$$\eta = \frac{T}{S} = \frac{Kb}{Ssb} \qquad (14)$$

$$\eta = Kb/Ssb \qquad (15)$$

$$\eta = k/Ss = T/S \qquad (16)$$

Where:

L: radial distance from pumping well to point of monitoring drawdown (L) (replacing L=r)
t: is time (T).
2 :Geometric term.
η : is aquifer diffusivity(L^2/T) which is equal to :

$\eta = (7.71*10^{-4})/0.16 = 0.005 m^2/s$

$r = 2\sqrt{Tt}$ (the equation based is based on the diffusion equation)

$r=2\sqrt{0.005*140.5*60}$

r=13 m (the distance from pumping well to monitoring well).
and Storativity:

$S' = \frac{2.25*0.0458*120}{13^2} = 0.086$
(which is reliable with the standard value)

The value of Storativity for unconfined aquifer type is within the range of (0.01-0.3) according to (Michael Kasenow,1997). the calculation is repeated for the other wells of (w-17, w-7, w-53, w-55, w-57, w-59) to verify the reliability of the results such as shown in Table 2:

Table (2) Transmissivity and Storativity Results

Well No.	Q m3 /d	SWL m	DWL m	B m	T m2/d	S
W-17	1600.56	82.7	106.3	182.3	66.6	0.037
W-7	1879.2	63	75	187	110.9	0.062
W-53	1458	49	81	201	35.11	0.019
W-55	1730.16	36	45	214	175.93	0.016
W-57	1697.8	50	61	200	97.1	0.054
W-59	952.56	55	70	173	69.74	0.039

As well as, the lithology profile of the selected well is shown in Figure below:

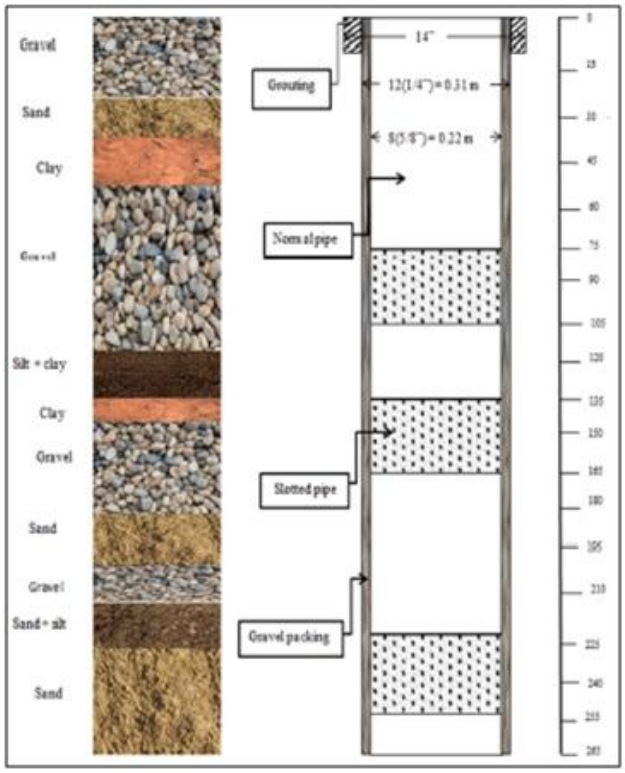

Figure 3: Lithology profile of the well.

3. RESULTS AND DISCUSSION

The presented method can be used to estimate the Storativity of the aquifer, according to the results, The value of Storativity such as shown in (Table 2) for unconfined aquifer is within the standard range of (0.01-0.3) according to (Michael Kasenow,1997). Therefore the study can satisfied that the values of the Storativity can be estimate from single well test by determining the radial distance from diffusivity equation then used to estimate the Storativity with the absence of the monitoring well, According to (Mawlood D. K 2019c), it is known that the values of Storage coefficient cannot find easily in case of the production well test due to the turbulent flow (well losses) in the vicinity of the testing well. The results of the study shows that the proposed method can be applied for the production well cases, that is satisfied based on the results obtained which are within standard limitations of the Aquifer parameters value as well. meanwhile this paper show that if the pumping and recovery test data available it can be substituted in the groundwater flow equations then find the Transmissivity value then Storativity can be found after calculating the distance form diffusivity equation in order to find the Storativity for the aquifer this can obtain the main goals of the study.

4. CONCLUSION

The presented study concluded that the method is more reliable for determining the aquifer Storativity for single well test which is a most powerful techniques because it is hard to find the Storativity in case of single well test due to the absence of the observation wells, so the paper presented a method to find the radius of the influence drawdown in order to achieve reliable and accurate results by applying diffusion principle for the distance measurement based on the pumping and Recovery test data the Storage coefficient can be estimated as well. this verified by finding the value which is within the standard range of Storativity for unconfined aquifer types which is between 0.01 to 0.3, (Michael Kasenow, 1997). thus the paper satisfied that the values of the aquifer parameters especially Storativity which can be find out from single well test by finding the radial distance through diffusivity equation and using this distance to calculate Storativity without existing monitoring well, however the application of this method can overcome the problems of the absence of the observation well and lead to reduce the cost of drilling observation well as well.

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RESEARCH PAPER

Grain Size Analysis as Paleo-environment Indicator of Lower Pliocene Sediments (Bai-Hassan Formation), Garmian Area, Kurdistan Region, NE Iraq

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A B S T R A C T:
Grain size distribution has utilized to determine the paleoenvironment sediments. The study is primarily dependent on the analysis and description of plots and interrelation factors between parameters. Five samples have been collected in the five different facies along (90) m thickness in the outcrop. The grain size is ranging from (Ø -8 to 14), (Boulder to Clay) which has given good attention to such research. Grain size analysis indicated the major of the particles are Gravel and the minor proportion is (Sand and Mud), which shows that the samples are more Gravely (> 80%) except for the sample (5) is less (< 50%). Sediment transport mechanisms indicated that bimodal sources and were moved associated with the bed (rolling, sliding, and salting) based on the force of the flow. The statistical parameters pointed out the obvious results of the sediment environment. The values of Median and Mean were negative which inferred the coarse pebble size, sediments were very poorly sorted, finely skewed, and leptokurtic. Interrelationship factors between the parameters showed a clear interpretation of the Paleoenvironment and streamflow. The indications have shown that the sediment deposits in a river environment, high current energy, accompanied by strong turbulence currents.

KEY WORDS: Keywords: Garmian, Grain size parameter; Paleoenvironment; Fluvial environment, Floods
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ZJPAS (2021) , 33(3);58-69 .

1.INTRODUCTION:

Grain size analysis is the fundamental mechanism determining the sediment because of the effectiveness of its techniques to discriminate and classify the sedimentary environment, and also provides significant details about transport history and precipitate conditions (Krumbein, 1938; Folk and Ward, 1957; Friedman, 1979; Tucker and Vacher, 1980; Blott and Pye, 2001). During recent decades, coarse-sized sediments have been greatly developed because of a widened attention in the environmental studies of the sediments, river depositions and surface processes (Lucchitta, 1978; Blair ,1987; Blair and McPherson, 1999).

The unconsolidated deposits are covering large areas in that location, with various grain sizes increasing to coarser in an area and finer in another. The sediments in the study area are a controversial topic, due to the difficulty in distinguishing between Bai Hassan and Mukdadyia formations (Jassim and Goff, 2006). The main aims of the study are; to determine and understanding the type of the Paleoenvironment trait, mechanisms of the sediment transportation, and the source of the materials transport processes by (water, air and land) (López, 2017). The grain size has been previously divided into millimeters (mm) and phi (Ø) units (Udden, 1914; Wentworth, 1922). The scale is divided into the range of the grain sizes from Boulders (Ø > -8) to Clays (Ø <8) as it showed in (Table 1).

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Table (1) Weight retained in sieve mesh, percentage, and the cumulative ratio of taken samples.

Sieve Mesh (mm)	Phi (Ø)	Sampl **1** (gm)	Wt%	Acc . Wt	Sample **2** (gm)	Wt%	Acc. Wt	Sample **3** (gm)	Wt%	Acc. Wt	Sample **4** (gm)	Wt%	Acc. Wt	Sample **5** (gm)	Wt%	Acc. Wt
256	-8	823	15.9	15.9	1190	36.5	36.5	1516	32.2	32.3	1750	33.0	33.0	1043	21.03	21.0
64	-6	941	18.2	34.2	420	12.9	49.5	1909	40.6	72.9	1282	24.2	57.3	534	10.7	31.8
16	-4	2255	43.7	78.0	428	13.1	62.6	203	4.32	77.2	294	5.55	62.8	350	7.06	38.9
4	-2	185	3.59	81.6	190	5.84	68.5	194	4.13	81.3	191	3.61	66.4	142	2.86	41.7
2	-1	240	4.66	86.2	90	4.31	72.8	193	4.11	85.4	207	3.91	70.3	450	9.07	50.8
1	0	170	3.30	89.5	170	5.23	78.0	150	3.19	88.6	380	7.18	77.5	685	13.8	64.6
0.5	1	90	2.72	92.2	180	5.54	83.5	143	3.04	91.7	277	5.23	82.7	490	9.88	74.5
0.25	2	55	1.07	93.3	103	3.17	86.7	114	2.43	94.1	238	4.49	87.2	320	6.45	80.9
0.125	3	108	2.10	95.4	100	3.08	89.8	48	1.02	95.1	170	3.21	90.4	380	7.66	88.6
0.0625	4	56	1.09	96.5	95	2.92	92.7	125	2.66	97.8	214	4.04	94.5	340	6.86	95.5
0.0039	8	143	2.78	99.3	142	4.37	97.1	84	1.79	99.6	188	3.55	98.0	130	2.62	98.1
Clay	14	35	0.68	100	94	2.89	100	20	0.43	100	105	1.98	100	95	1.92	100
Total (Kg)		5,151	100		3,252	100		4,699	100		5,296	100		4,959	100	

Statistical parameters are generally reflecting the grain size distribution data and show insight features of the sediment environment and also can be considered a good proxy of different grain sizes (Folk, 1974). Median and Mean may be easily calculated but they do not represent the extremes of the curve, while the other parameters (Standard deviation, Skewness, and Kurtosis) demonstrate the ratios of dispersion and show a wide range of understanding and interpretation about the environment system (Folk, 1974).

Inman (1949) mentioned three essential modes of particle movement which are rolling, salting and suspension. The sediment particles are rolling connecting the bed and moving intermittent neither steady nor continuous. The coarse-sized particles such as gravels and coarse sands are rolling or sliding, knowing as surface creep or traction load (Visher, 1969). Determination of sedimentary environments, grain size analysis with its parameters have been applied on the various facies of the samples. Most of the selected facies are rich in gravel, however, some are containing varying quantities of sand, silt, and clay. Samples are located in the Garmian area, nearby the Serwan river (Figure 1).

2. GEOLOGICAL SETTING

The study area is located in Garmian area, between latitudes ($34^{o}51'\ 26 - 34^{o}\ 50'\ 14''$), and longitudes ($45^{o}32'\ 32'' - 45^{o}\ 31\ 02''$), South of Sulaimaniyah Government, North East Iraq (Figure 1). Structurally, it is located in an unstable shelf particularly in the Foothill zone, on Serwan transversal Fault (Jassim and Goff, 2006) (Figure 2). A fault is extending along the Serwan river and prolongs from NE-SW of Iraq. The area is full of deep synclines filled with Pliocene deposits in coarse molasses type such as conglomerates.

Geologically, the sediments in the area are including between recent sediments, Bai-Hassan and Mukdadyia formations (Lower Pliocene), Injana Formation (Upper Miocene), and Fatah (Lower Fars) formation (Middle-Lower Miocene) Figure (2). Quaternary sediments are adjacent Serwan river and covering the floodplain and riverbank. These formations are often composing of gravel, sand, and clays. The study area has not studied yet and is not easy to distinguish between two main formations in the same range of age, which are Bai- Hassan and Mukdadyia formations. These two formations have the same compositions sway between cycles of gravel, gravely sandstone, sandstone, and mudstone, however, they can be recognized by their distribution in the Foothill zone and high folded zone (Jassim and Goff, 2006).

According to Jassim and Goff (2006), during Late Miocene-Pliocene, the Arabian plate collided the Iranian plate led to lifting areas, by its role, formed High folded zones, north Iraq. During the Late Miocene-Pliocene, conglomerate and sand-sized grains have been settled down as a result of weathering and the uplift occurred later in Pliocene. The study area consists of conglomerate, sandstone and clays. Distribution of geological depositions and different types of faults. It is particularly appearing Foothill zone sediments, north Iraq (Figure 2).

3. METHODOLOGY

The samples have collected in an outcrop beside the Serwan river, approximately (90 m) thickness, which appeared its exposure to erosion (Figure 1). One site has selected among six

different locations that previously visited, about (5Kg) were collected for each sample (Table 1).

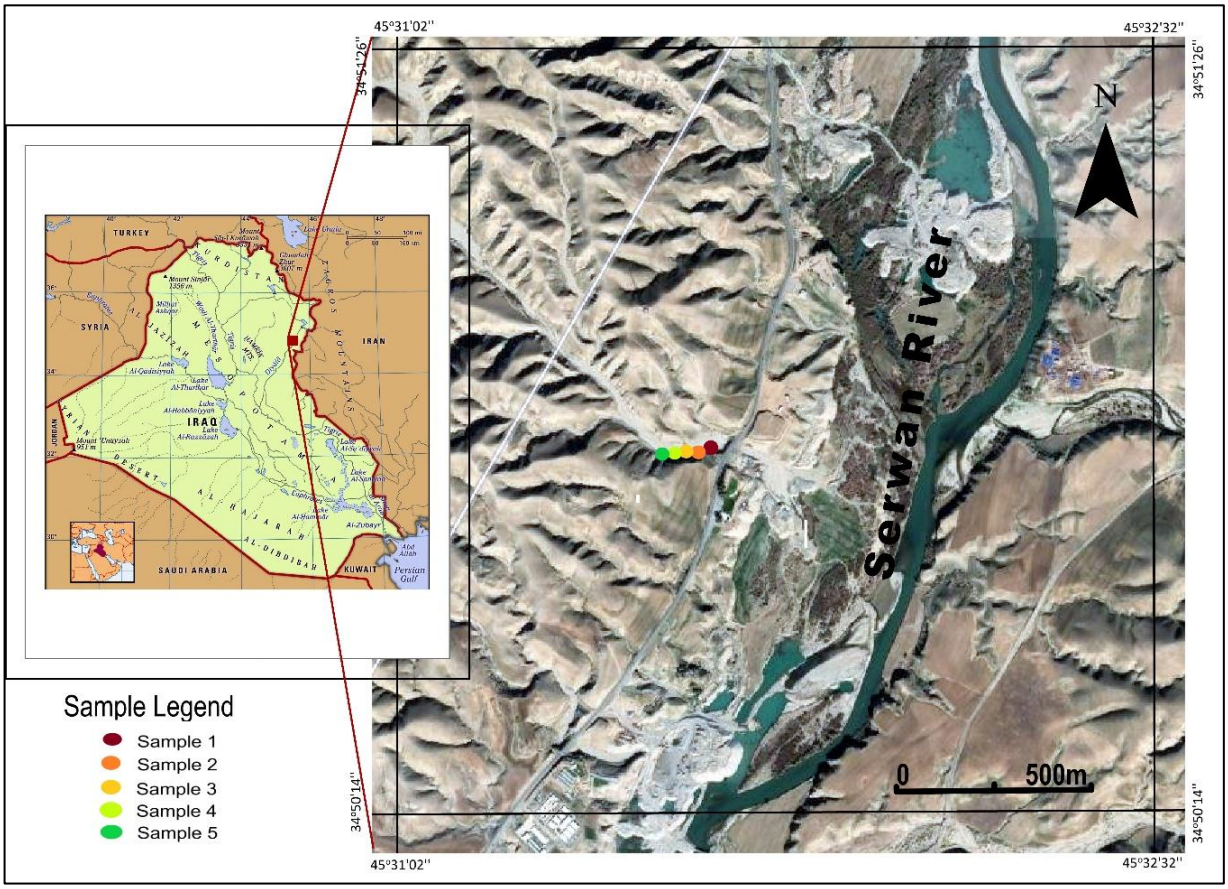


Figure 1: Google Map of the study area (Garmian, NE Iraq) explaining the sample locations.

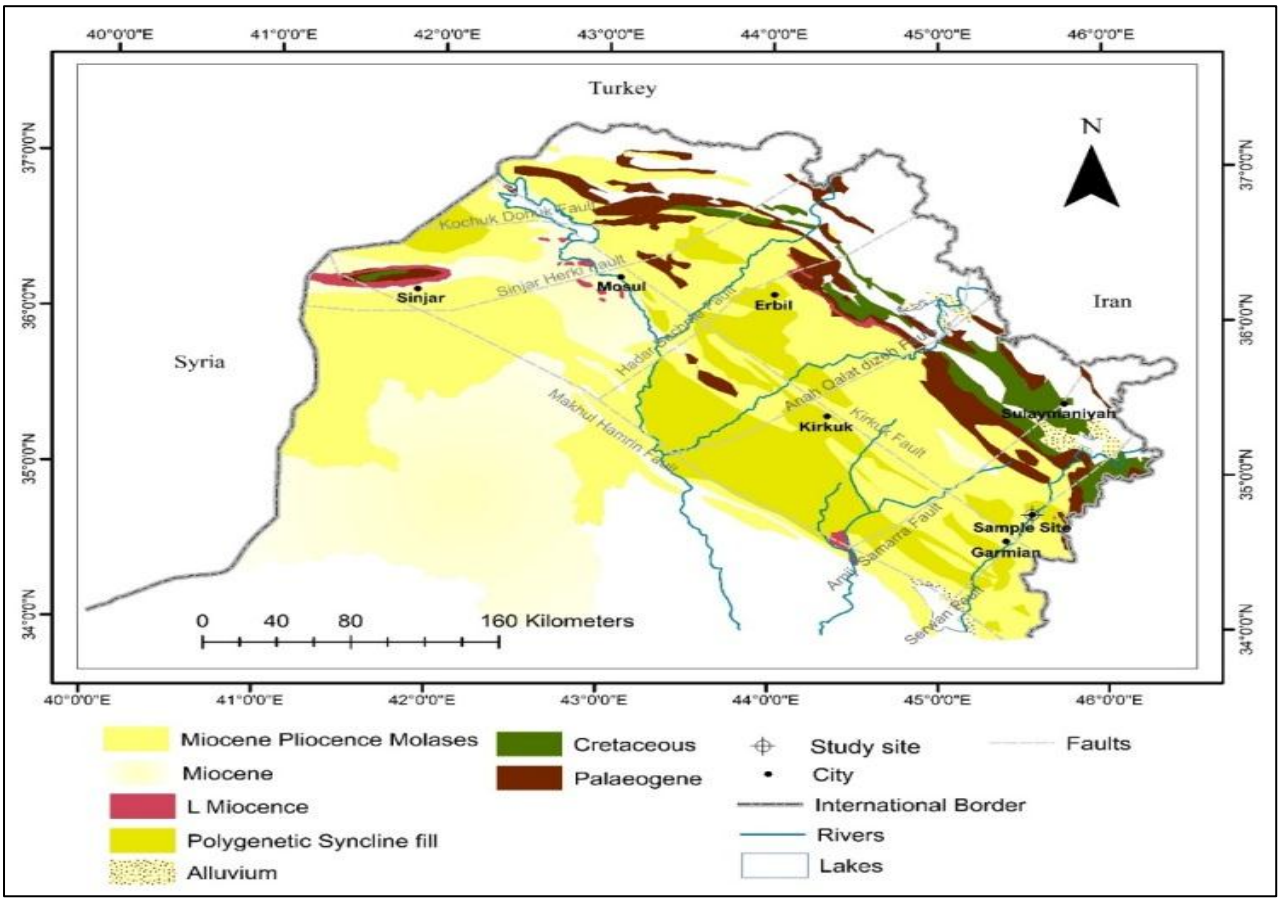


Figure 2: Geological map of North Iraq, showing the samples site and the main faults (normal and reverse) (after Jassim and Goff, 2006).

The study built upon two main methods which are: direct description in the field interpretation by grain situation, graduation and orientation based on the sedimentary structure such as packing grains, and also Lab analysis of the samples. Aggregate samples have been dealt chemically and physically to break up into particles. Samples analyzed in Garmian Engineering Testing Laboratory (GETL) using American Society for Testing and Materials (ASTM) Sieves, Vibrator and weight-sensitive balance.

Folk's grain size parameters still have a wide range of use among the geological milieus, as shown in the equations and been applied in (Table 2 and 3). The equations are:

Table (2) Percentage proportion of gravel, sand, and mud of the samples.

Sample No.	Gravel %	Sand %	Mud %	USDA
1.	86.2	10.3	3.5	Muddy Sandy Gravel
2.	72.8	19.9	7.3	Muddy Sandy Gravel
3.	85.5	12.3	2.2	Muddy Sandy Gravel
4.	70.4	24.1	5.5	Muddy Sandy Gravel
5.	50.8	44.7	4.5	Sandy Gravel

Percentage Retained (%): $\frac{Ws}{Wt} \times 100$…… (*Equation-1*)

Where, *Ws* denotes weight retained in a sieve, *Wt* the total weight. The percentage ratio for gravel, sand and clay, separately calculated for each sample (Table 2) and was applied to the Folk's Triangular plot (Figure 3). The plot showed that the grain size distributed between gravel, sandy gravel, and muddy sandy gravel.

$M_d = Ø_{50}$ …….. (*Equation-2*) (Folk, 1974).

The equation built upon the idea half of the particles is coarser by weight than the Median, and half is finer.

Mean (Mz)

$(Mz) = \frac{Ø_{16} + Ø_{50} + Ø_{84}}{3}$ …… (*Equation-3*) (Folk, 1974).

Inclusive Graphic Standard Deviation (Sorting) (σ_1)

(σ_1) ——— ——— …… (*Equation-4*)

(Folk and Ward, 1974)

< Ø 0.35 Very well sorted
Ø 0.35 to Ø 0.5 Well sorted
Ø 0.50 to Ø 0.71 Moderately well sorted
Ø 0.71 to Ø1.0 Moderately sorted
Ø 1.0 to Ø 2.0 Poorly sorted
Ø 2.0 to Ø 4.0 Very poorly sorted
> Ø 4.0 Extremely poorly sorted

Inclusive Graphic Skewness (SK_1)

(SK_1) =——————— ———————…… (*Equation-5*)

(Folk, 1974)

Ø 1.0 to Ø 0.3 Very fine skewed
Ø 0.3 to Ø 0.1 Fine skewed
Ø 0.1 to Ø -0.1 Near symmetrical
Ø -0.1 to Ø -0.3 Coarse-skewed
Ø -0.3 to Ø -1.0 Very coarse skewed

The Graphic Kurtosis (KG_1)

(KG_1) =———————……. (*Equation-6*)

(Folk, 1974)

< Ø 0.67 Very Platykurtic
Ø 0.67 to Ø 0.90 Platykurtic
Ø 0.90 to Ø 1.11 Mesokurtic
Ø 1.11 to Ø 1.50 Leptokurtic
Ø 1.50 to Ø 3.00 Very leptokurtic
> Ø 3.00 Extremely leptokurtic

These equations undergo to mathematical and statistical processes to be presented later on graphs and plots. The mean is illustrating grain size distribution of the materials and also reveals the stream energy, depending on the velocity and turbulence of the transporting medium (Folk, 1980). Sorting is a function of the grain size range of the sediments and reflects the amount of energy, and flow velocity by its role, steadiness in the current energy, in somewhat, states a good sorting (Al-Miamary, 2000). Skewness and kurtosis show how closely the grain-size distribution approaches the normal Gaussian probability curve, and the more extreme values, the curve will be non-normal (Folk, 1974).

4. RESULTS AND DISCUSSIONS

The research is used different types of plots, graphs, and statistical parameters to come into precise results. To be fairly explained, it requires extra details. Therefore, the discussion examined three main results which are frequency curves, grain size parameters, and interrelationship factors between the parameters.

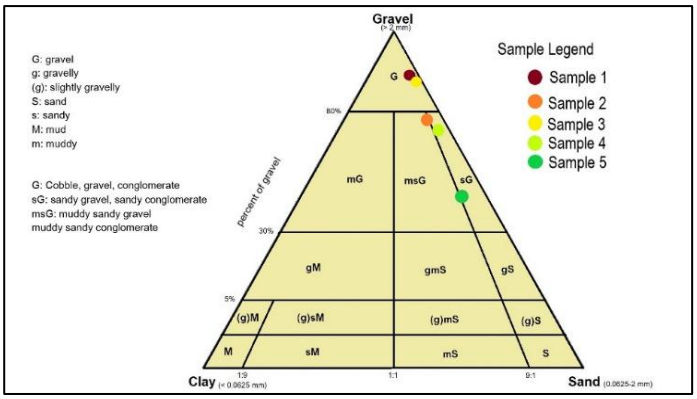


Figure 3: Triangular diagram showing the sieve analysis of the samples (Folk, 1980).

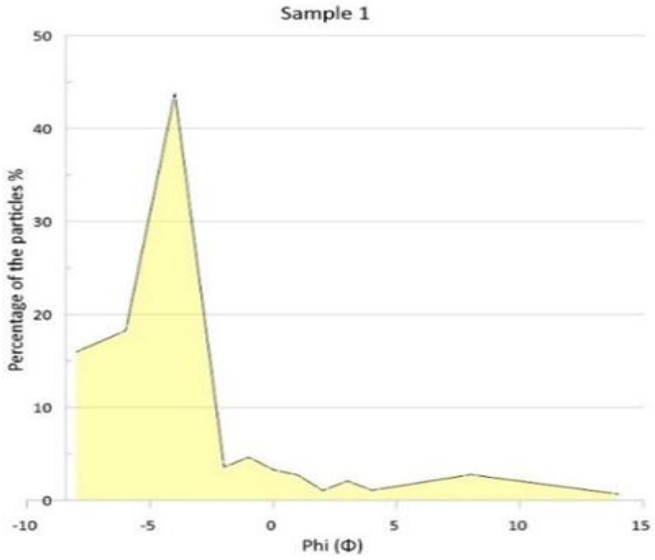
4.1 Frequency Curves

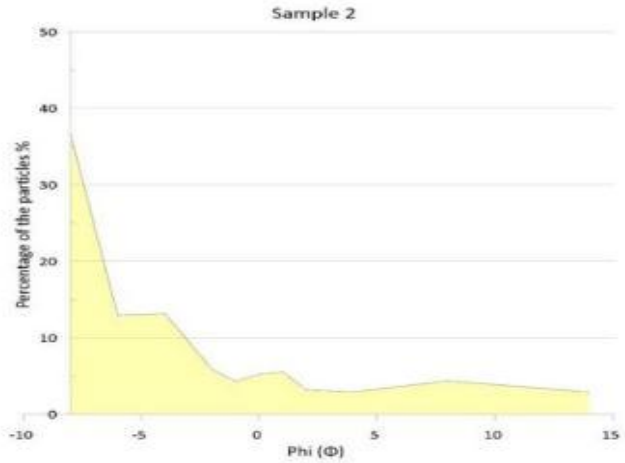
Twenhofel and Tyler (1941) importantly mentioned the statistical processes for grain size allowing immediate and easy comparison of coarse-sized sediments and implies the similarities and differences in terms of sediment environmental conditions. The percentage ratio of grains has calculated for each sample (Table 2) by (*Equation* 1). The Folk's ternary diagram revealing distributed between Gravel (>70%), Sand (<20%), and Mud (<5%) (Figure 3). The ternary diagram illustrated that the common particles are cobble, conglomerate, sandy conglomerate, and muddy sandy conglomerate. The proportion of cobble and gravel states the high flow velocity of the stream at the sedimentation.

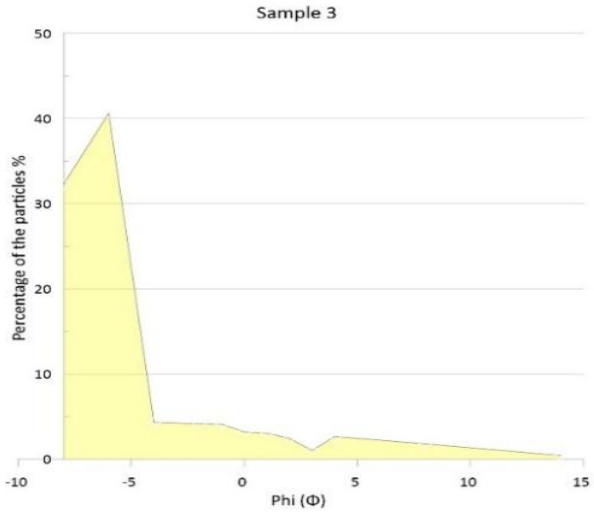
The size-frequency curve denotes the natural distribution of sediment and providing more details on the sorting, symmetry, and modality index (Friedman and Johnson, 1982). The frequency curve indicates that the sediment modality is a bimodal distribution attributing that it is derived from more than a source, and irregular kinetic energies in the depositional environment (Figure 4). However, it can be assumed that there are some changes in kinetic energy. The changes are not limited to the sediment environment, but rather extend to the movement of particles during the flood (Folk and Ward, 1957). In addition to the presence of variation in the grain size, there are sudden changes have been occurred, perhaps because of branching and converging river channels (Aghwan and Al-Fattah, 2005).

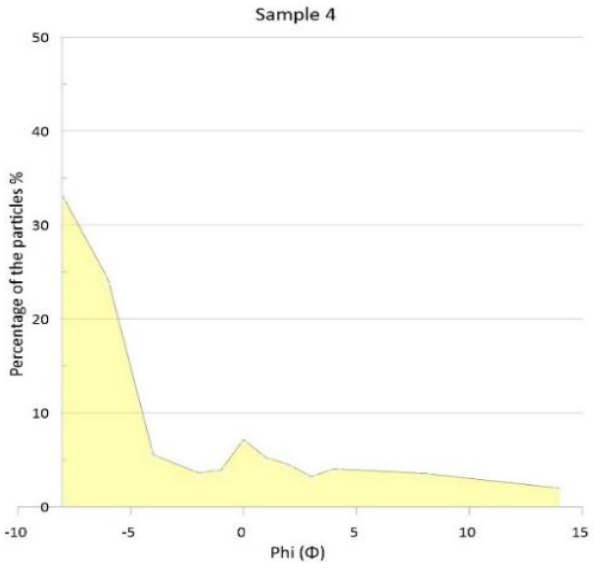
Einstein *et al*., (1940) used the relationship between grain size diameter (Phi) and the accumulative weight of the grain size in which explains the transport mechanism of the sediments. The total sediment load is divided into bed material load and washload (Konodolf and Piegay, 2016).

The bed material load derived from Coarse-Sized Grains and the washload typically derived from Fine-Sized Grains, both of which have flushed into the stream from the upland sources. Based on the figure (5), most of the sediments are considered bedload materials which including cobble, pebble, and coarse sand grains. By mode of transport, the sediment load is often moving in attaching along the bed, whether rolling, sliding, or salting (samples 1, 2, 3). However, some of the particles are seemed as washload materials and transported in the mode of suspended which dispersed in the flow by turbulence and carried suspending from long distances (samples 4, 5). The movement of the particles can be classified:









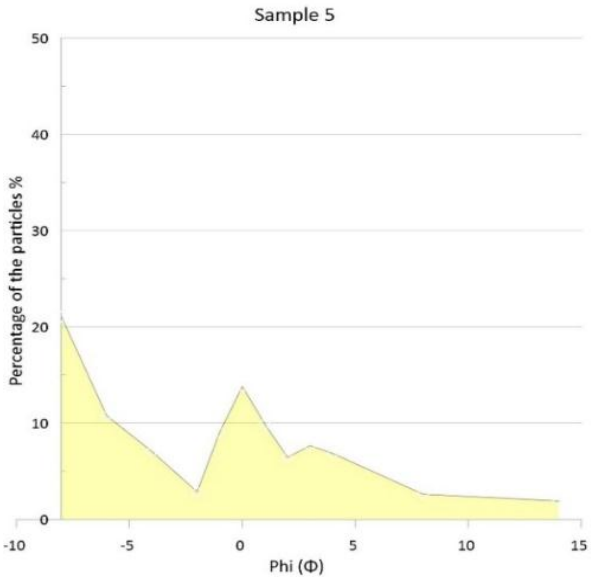
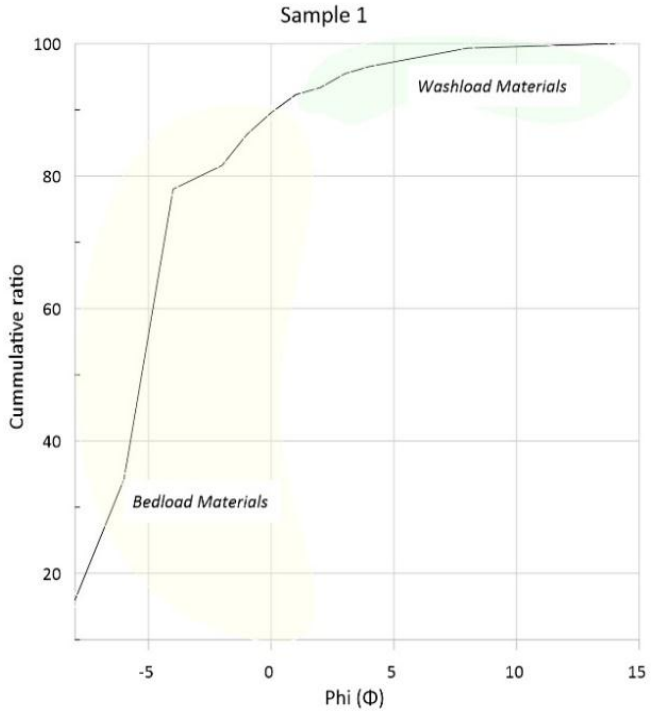
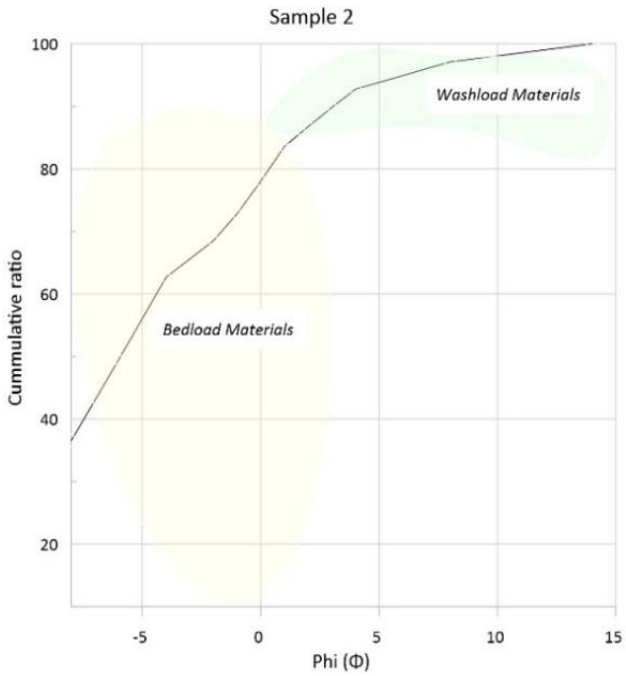


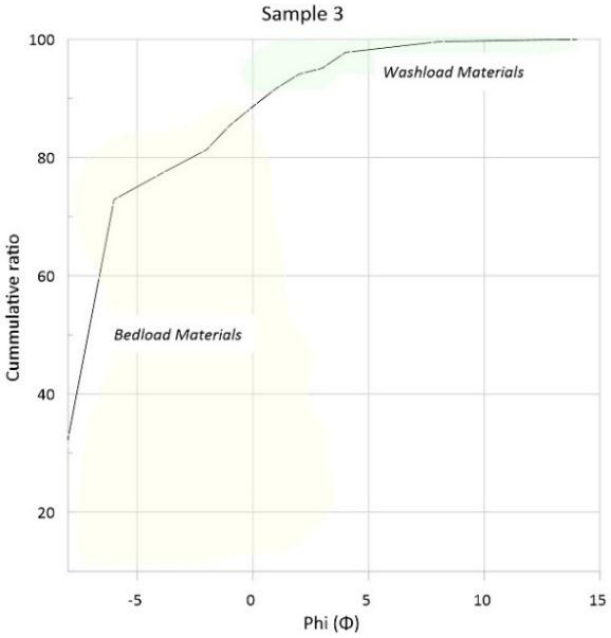
Figure 4: Frequency Curve distribution of the Samples (Folk, 1980)

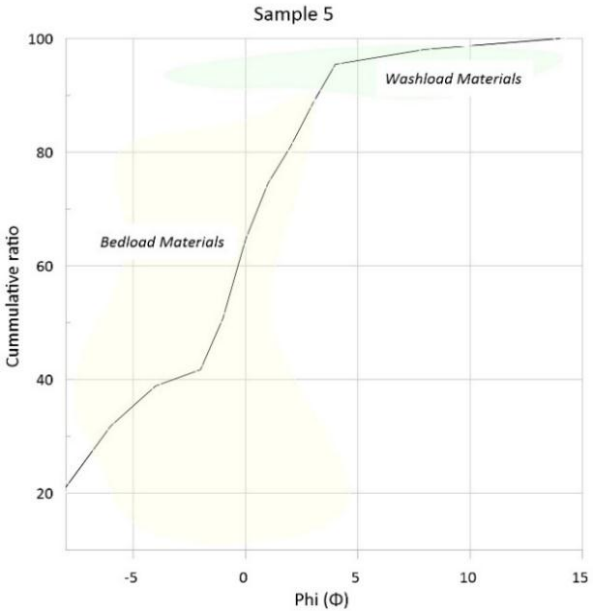
a. *Rolling transport:* appearing within the coarse- sized material ranging (Ø -8 to -4) in most of the samples, except sample (5). This states the sediment movement (rolling or creeping), which often occurs because of variation pressure on the grain, reverse to the stream direction (Passega and Byramjee, 1969) (Figure 5).
b. *Saltation transport*: included from (Ø -4 to 4), it takes a long inclined crouch in the curve (Figure 5). This type of movement may occur in the flash flood and represents the movement of the graded bed and exists above bedload materials (Passega and Byramjee, 1969).
c. *Suspension movement*: including the fine-sized materials, ranging between (Ø 4 to 14) which is covering (very fine sand, silt, and clay) by less than 5% of the total percentage and appear as a straight line in the curve (Figure 5). However, this class is representing the equilibrium status between the forces that generate turbidity against the stability directions of the grains (Sagoe and Visher, 1977).

The presence of grains is ranging between phi (Ø -8 to 0) which might indicate high kinetic energy, flooding successions, and the fluvial environment. Certain changes have appeared in the cumulative frequency curve, particularly (sample 5), which can be considered turbid currents or disturbances in the current energy during sedimentation.









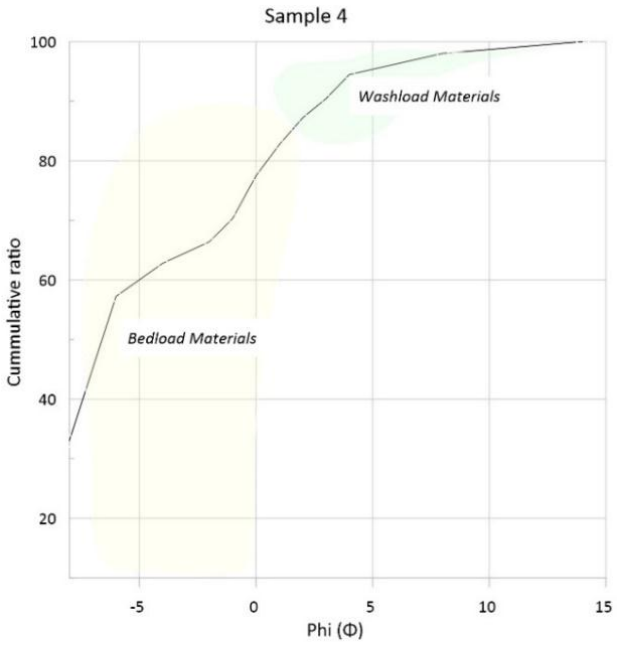


Figure 5: Cumulative Frequency Curve distribution of the Samples (Folk, 1980)

4.2 Grain Size Parameters

These parameters are more accurate and attain satisfying results including:

1. *Median (Md)*
   The median is the relative value separating the fine part from the coarse part (Folk, 1974). The median values varied in the study ranging (Ø-1.2 to – 7.2), (equation 2), on average (Ø-5.2) giving humble results on the relative coarse-sized grain of the samples (Table 3).
2. *Mean (Mz)*
   It is a function of primary grain size for the source rocks giving the ability of average deposit in the sediment (Pettijohn *et al*., 1973). and an important factor of depositional environments (Friedman, 1967) Mean's result is more accurate than the median because the mean depends on three points of grain size measurements and gives a preferable portrait of the sediments (Folk, 1980). The mean has shown (Ø-3 to -4.9), (Equation 3) on average (Ø -4.2) and the biggest assemblage values at (Ø -4) (Table 3). This indicates that the grains are ranging from (Pebble to Granule) which concluding the sediments are transported from relatively the near distances, and to some extent lead to an increase of particle size near the source. It is evidence of high current

energy and a characteristic of floods and fluvial sediments (Reinck and Singh, 1980).

3. *Standard Deviation (Sorting)* (σ_1)

Sorting referred to the degree of concentration or dispersion between the grains. In the fluvial sediments, sorting is the process by which grains of a given size are populated. Well-Sorted means the grains are in identical sizes but poor-sorted presents the grains are in different sizes (Konodolf and Piegay, 2016). Standard deviation measures the sorting of sediment displaying the changes in the kinetic energy condition. When the sediments are reflecting different modes, the standard deviation is recording the variation in the kinetic energy, turbulence measures of poorly sorted sediments (Sahu, 1964). The standard deviation (σ1) results are between (2.9-4.4), (equation 4) on average (3.8) that means most of the samples are very poorly sorted to extremely poorly sorted, the most frequency value of sorting is extremely poorly sorted while the average value focusing on very poorly sorted (Table 3). The kinetic energy of the river can be overall considered irregular and high-velocity flow.

4. *Skewness (Sk1)*

It is used to measure the asymmetry of the frequency distributions and the position of the mean concerning the median (Sahu, 1964). Once the value of skewness is positive the sample is considering fine-skewed, which the Mean tends to the finer side of the Median. When the value of skewness is negative, the sample is coarse-skewed, and the mean tends to be the coarser side of the median (Sahu, 1964). Its significance came when it measures the environmental factor (Hills, 1967). The Skewness values are positive except sample (5), (Table 3). These results are demonstrating that the skewness values are very fine-skewed with average fine skewed. (Brambati, 1969) in (Maity and Maiti, 2018) explaining the positive skewness values is an indication of a one-directional of the streamflow. Mentioned (Valia and Cameron 1977) that the positive value is an indicator of the transportability factor and unidirectional flow.

5. *Kurtosis (KGi)*

It is considered a sensitive measurement of the nature of grain size distribution, and it defines as a measurement of peakedness of the distribution (Folk and Ward, 1957). The Kurtosis values of the samples ranged between (0.588-2.869), (Equation 6), (very platykurtic to very leptokurtic) in an average of (1.433), (leptokurtic), (Table 3). It can be said that the changes in the values of kurtosis are closely related to the determination of the origin of the sediments by identifying their models, and most of the sediments have one source and this matches the interpretations of the frequency curve (Cronan, 1972).

4.3 Interrelationship Factors

Using different statistical parameters gives a specific conception of the sediment environments, as well as the reflection of the transportation processes with the flow conditions that surrounded by the sediment environment (McLaren and Bowels 1985; Ghosh and Chatterjee 1994). Parameter values are indicating the nature of the current energy and flow condition of the sediment environment. Positive skewness represents the unidirectional flow of the stream (Brambati, 1969). Baruah *et al*. (1997) pointed out that high or low values of kurtosis reveal that part of the deposition is sorted in a high energy environment.

In order to come to delicate indications on the transport process, flow condition, and sedimentation, interrelation factors between available parameters have been applied. These factors are a good way to recognize such various facies to reach sedimentation conditions. (Figure 6.a) illustrating the relationship between Mean and Standard Deviation. The studies have mentioned such a relationship as an indicator between sediment environments (fluvial system, beach, and aeolian deposits) (Friedman, 1961, 1967; Moiola and Weiser, 1968; Folk, 1966, 1980; McLaren and Bowels, 1985; Al Rashedi and Siad, 2016). The relationship is shown that all the samples are ranged between a coarse pebble and granule grain size, within the scope of the river or fluvial sediments that reinforcing the previous analysis.

The relationship between Skewness and Standard Deviation (Figure 6.b) implies the fluvial environment of the samples. The plot is previously studied (Moiola and Weiser, 1968; Folk, 1966,

1980; Camerlenghi *at. el.*, 1995; Blott and Pye, 2001; Saeed and Siad, 2016), and came out to that all the samples distributed between extremely poorly sorted and very poorly sorted that situated within the fluvial environment outline.

Table (3) Results of the different parameters applying on the standard measurements

Sample No.	Median *(Md)*	Mean *(Mz)*	Standard deviation *(σ1)*	Skewness *(Sk1)*	Kurtosis *(KGi)*	Mean	Standard Deviation	Skewness	Kurtosis
1.	-5.2	-4.9	2.9	0.303	1.581	Coarse Pebble	Very Poorly Sorted	Very Fine Skewed	Very Leptokurtic
2.	-6.0	-4.6	4.4	0.613	2.869	Coarse Pebble	Extremely Poorly Sorted	Very Fine Skewed	Very Leptokurtic
3.	-7.2	-4.5	3.2	0.804	1.448	Coarse Pebble	Very Poorly Sorted	Very Fine Skewed	Leptokurtic
4.	-6.5	-4.0	4.1	0.738	0.681	Coarse Pebble	Extremely Poorly Sorted	Very Fine Skewed	Platykurtic
5.	-1.2	-3.0	4.4	-0.248	0.588	Granule	Extremely Poorly Sorted	Coarse-Skewed	Very Platykurtic
Average	-5.2	-4.2	3.8	0.248	1.433	Coarse Pebble	Very Poorly Sorted	Fine Skewed	Leptokurtic

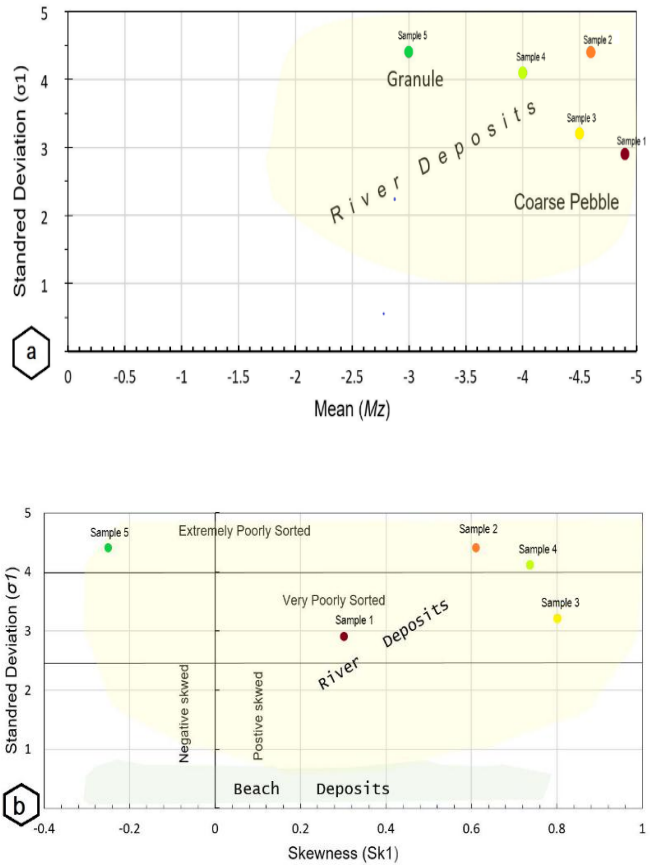


Figure 6: Interrelationship between different parameters showing the sediment environment. (a) the relationship between Standard Deviation and Mean. (b) the relationship between Standard Deviation and Skewness. (c) the relationship between Kurtosis and skewness (Maity and Maiti, 2018).

Demonstrating the relationship between Skewness and Kurtosis (Figure (6.c)), might be used as an indicator of the environment. The samples positively skewed except for (sample 5). On the other side, the samples ranged within very leptokurtic to very Platykurtic ranges. The results referred that the sediment accumulated in the foredeep basin coming from the high folded zone

and thrust belt through several sudden progressions. The settling has come from the disparity in topography and sediment load which observed the visualize of the Bai Hassan formation environment is fluvial condition and far away from other environments such as Delta and Beach.

5. CONCLUSIONS

The study dealt with a few sedimentary aspects regarding paleoenvironmental determination using grain size analysis. Various statistical parameters have used such as Median, Mean, Standard Deviation, Skewness, and Kurtosis with their interrelation factors. The study came out that the sediments deposited in the fluvial environment during rapidly flooded transgressions, associated with high current energy and turbulence, uni-streamflow direction, and bimodal sediment. Deposition materials filled the synclines during the rapid advances and catastrophic floods during the lower pliocene.

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RESEARCH PAPER

BACTERIAL PROFILE, ANTIBIOTIC RESISTANCE PATTERNS, AND ASSOCIATED FACTORS AMONG HEMATOLOGICAL MALIGNANT PATIENTS IN ERBIL CITY

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A B S T R A C T:

Background and Objectives: Cancer are one of the major global health problems**,** patients with hematological malignancies are highly susceptible to almost any type of bacteria. In this study, we aimed to determine the bacterial profile, antimicrobial resistance pattern, and associated factors among patients with hematological malignancies.

Method: A consecutive 70 hematological malignancies patients were enrolled from January to October, 2020; at Nanakaly hospital for blood disease and cancer in Erbil- Kurdistan region. Clinical data and Socio-demographic were collected by a structured questionnaire. Culture and antibiotic resistance were performed following standard microbiological procedures.

Result: The incidence of bacterial infection among examined cancer patients were 62.8 % (44/70). *E.coli* was among the predominant bacterial isolates (20%), followed by *Klebsiella pneumoniae* (11%), *Streptococcus parasnguinis*, and *Staphylococcus haemolyticus* (9%). These bacterial isolates were resistant to different antibiotics. Gram- negative bacteria were highly resistant to several antibiotics including, ciprofloxacin, gentamycin, sulfamethoxazole/trimethoprim, piperacillin, and cefepime, but they were sensitive to imipenem, amikacin and tigecycline. While gram-positive bacteria were highly resistant to tetracycline, ceftriaxone, levofloxacin, ampicillin, and erythromycin, but they were highly sensitive to linezolid, tigecycline, imipenem and vancomycin. The majority of resistance organism against fluoroquinolone were *E.coli* (72.7%) followed by *Klebsiella pneumoniae* (66.7%). Among gram-positive bacteria *Streptococcus parasnguinis* was highly resistance while sensitive organisms were *Staphylococcus aureus*, CoNS, *viridance streptococcus* and *Enterococcus faecalis.*

Conclusion: Among hematological malignant patients, the majority of patients were diagnosed as acute leukemia, gram-negative bacteria were more frequently isolates comparing with gram- positive bacteria. *E coli* was among predominant pathogen followed by *Klebsiella pneumoniae*, *Streptococcus parasnguinis* and *Staphylococcus haemolyticus*.

KEY WORDS: Bacterial profile, antibiotic resistance pattern, Hematological malignancy.
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ZJPAS (2021), 33(3);70-84.

1. INTRODUCTION:

Cancer are one of the top global health and hygiene matters after cardiovascular diseases, major crises, and traumatic accidents worldwide. Cancer caused around 13% of all human deaths globally (Abdollahi *et al.,* 2016). More than half of all cancer cases and about 60% of deaths occur in developing countries (Qin *et al.,* 2007). Approximately 9 million people worldwide died by reason of complications from cancer, and the incidence is expected to double by 2030 (Ferlay *et al.,* 2015). There are many types of cancers classified in solid tumor and hematological tumor, the incidence and risk factors are in continuous change depending on the personal and environmental factor (Bray *et al.,* 2018).

Better care for cancer patients over the past several decades have significant improvement in patients survival (Fentie *et al.,* 2018a). Patients with cancer are susceptible to serious

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complications, among them infections were responsible for substantial increment mortality and morbidity (Fentie *et al.,* 2018a). Patients with cancer who are undergoing chemotherapy are highly susceptible to almost any type of bacterial infection (Vento and Cainelli, 2003). Cancer patients obtaining infection of bacterial either through exogenously from the hospital environments or endogenously from normal flora near the operative sites (Nurain *et al.,* 2015).

The pattern of bacteria causing infection in patients with cancer have altered over the past decades(Kanamaru and Tatsumi, 2004). Gram-negative bacteria were detected as the common microorganism in1970. However, gram-positive bacteria became the predominant microorganism in1980 (Rasmy *et al.,* 2016). The incidence of gram-negative bacteria increased throughout the last two decades in an adult, whereas in pediatric patients, an increase in gram-positive bacteria was detected. In cancer patients, particularly during treatment with chemotherapy agents, most cells of the human body undergo undesirable changes which cause disruptions of bodies' defense system. Moreover, exposures to potentially pathogenic microorganisms increase as result of the frequent use of invasive procedures beside the empirical use of broad spectrum antibiotics (Meidani *et al.,* 2013).

The infection of patients with hematological malignancy may affect any organ most commonly affecting the respiratory tract, urinary tract, blood, and skin (Azoulay and Schlemmer, 2006) (Lagier *et al.,* 2016).

The susceptibility of microorganisms for antibiotic also changes with the period, with the emergence of multidrug-resistant organisms since the discovery of antimicrobial agents, microorganisms have developed resistance to them through mechanisms such as mutations and increased enzyme production (Anderson *et al.,* 2012). Resistance to commonly used antibiotics is an important problem worldwide (Graffunder *et al.,* 2005). Infection pathogens with multidrug-resistant (MDR) counting extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are most predominant among cancer patients (Biehl *et al.,* 2016). Enterobacteriaceae cause around 65%–80% of documented gram-negative infections in cancer patients (Saghir *et al.,* 2009). Patients profile, environmental factor and geographical distribution were mainly responsible for emerging resistant pattern among cancer patients (Versporten *et al.,* 2018) (Tsutsui and Suzuki, 2018). Therefore a periodic epidemiological data and molecular studies are necessary for knowing the pattern of microbial resistance which guides the clinician for better and effective use of empirical therapy and better infection (Lebea and Davies, 2017). The aim to this study was to determine the bacterial profile, antimicrobial resistance pattern, and associated risk factors among patients with hematological malignancy.

MATERIALS AND METHODS

Study Population and Sampling

This is a cross sectional prospective study done at the Nanakaly hospital for blood disease and cancer in Erbil city- Kurdistan Region. Seventy cancer patients (39 males and 31 females) were include in this study from - January 2020 to October 2020; patient were provided with informed consent. This study was achieved according to the ethical committing at Hawler Medical University. Cancer patients were selected after an interview through a structured questionnaire designed for such purpose, which consists of three parts including, demographic information: include (age, gender, occupation, marital status, level of education), patients statue and cancer types as well as types of treatment that patient receives. A total of 144 clinically related specimens were collected from 70 patients diagnosed with hematological malignancy, specimens were collected from different clinical site including; sputum, blood, urine, wound swab, throat swab and stool.

Blood sample processing and collection:

Blood samples (8–10 mL blood from a patient) for cultures were taken from each patient who developed a fever at the time of diagnosis. Blood samples were drawn from two different sites of peripheral vein aseptically (disinfecting with 70% alcohol and 2% tincture of iodine .which are used for blood cultures by experienced nurses prior to any antibiotic use) (Little *et al.,* 1999) (Calfee and Farr, 2002). Samples were immediately transferred into the Automated BacT/ALERT3D (bioMerieux, France) system, plus blood culture bottles (Doern *et al.,* 2014). These bottles were specifically designed to

provide rapid and sensitive detection of micro-organisms (bacteria and yeast), when only a small volume of blood was available. They contained a smaller volume of broth, thereby still providing an optimal blood to broth ratio when a limited volume of blood was collected. The BacT/ALERT® PF Plus blood culture (bioMerieux, France) system, could accommodate up to 4 mL of blood (Doern *et al.,* 2014). As such, it was appropriate for blood cultures from hematological malignancy patients, bottle were labeled with identification number, date, gender and age. Then it was taken into BacT/ALERT3D 60 remarkably complete by recording barcodes, all blood cultures have been observed for at least one week to appear either infectious or noninfectious samples of blood by their screen (Doern *et al.,* 2014).

Urine Sample Collection and Processing:

Midstream urine was collected with a sterile urine container from both symptomatic and asymptomatic urinary tract infection (UTI) cases. Standard wire loop (0.001/ mL) used for taking specimen for culture. Specimen were inoculated on MacConkey agar and 5% sheep blood agar. All sample were incubated at 37°C for 18–24 hours. Significant bacteriuria was defined as a colony count ≥105 CFU/mL urine (Fentie *et al.,* 2018b).

Wound /Throat swab Processing and Collection:

Wound and throat swab samples were collected aseptically based on the clinical manifestations of the cancer patients by using a sterile saline moistened cotton swab. The swabs were streaked on MacConkey agar, blood agar plates, and Mannitol salt agar. These plates were then aerobically incubated for 18–24 hours at 37°C (Fentie *et al.,* 2018b).

Sputum Collection and Processing

Sputum samples in the early morning were collected from each patient, the expectorated specimen was placed in a sterile petri dish .through the use of a sterile wire loop 0.001ml, specimens were cultured on blood agar medium incubated aerobically at 37°C for 48 hrs (Bartlett and Finegold, 1978).

Specimen cultivation

Different types of culture media including: MacConkey agar, Mannitol salt agar, blood agar and Eosin Methylene Blue (EMB) were used to obtain a pure isolates. The urine specimens were inoculated on the plates of (EMB) and MacConkey agar and then distributed on the surface of plate for incubation, to obtain a single colony. After incubation the pure isolated colonies were tested for colonial morphology and lactose fermentation. The wound and throat swabs were cultured on blood agar, MacConkey agar and Mannitol salt agar and incubated. After incubation the pure isolated colonies were tested for colonial morphology. All the media were incubated at 37°C for 18–24 hours. For further identification of culture pathogen other tests have been performed including, test describing morphological characteristics and biochemical characteristic of isolation pathogen (Elmanama *et al.,* 2013).

Identification Technique of the Isolated Bacteria

Sub-Culture of the Isolated Bacteria:

Sub-culture of single colony of isolated bacteria was achieved by inoculating single colony into the plate by quadrant method. The inoculated plates were incubated at 37 ˚C for 24 hrs. And then the growth spread over a wide area (Ge and Taylor, 1997). Isolation, identification and purification of isolated bacteria by traditional methods depend on cultural, morphology of bacteria, such as hemolysis phenomenon, and lactose fermentation ,identification of bacterial shape one according to the result from Gram stain method (Atlas, 2010).

Gram Staining:

Is the first step of bacterial identification, a smear of bacteria was deposited on a glass slide and carefully air-dried, then after stained for 1 min in Crystal Violet solution, 1 min in an iodine solution, washed for 10 sec. in ethanol, and finally, counterstained with safranin for 1 min. The glass slide examined under oil immersion under light microscope (Pandolfi and Pons, 2004).

Culture Media:

Nutrient Agar Medium

This medium was used for preservation of pure culture on slant. It also useful for detect of pyocyanin (water soluble bluish-green pigment) production by *Pseudomonas aeruginosa (Atlas, 2010).*

Blood Agar Medium

Blood agar is frequently used as a universal enrichment medium. Most human bacterial pathogens grow on blood agar. It is prepared by autoclaving blood agar base at 121 °C for 15 minutes, after cooling to 45-50 °C. 50 ml of sterile defibrinated human blood were added aseptically

to 1 liter of the medium, mixed thoroughly and poured into sterile Petri dishes (Atlas *et al.,* 1995).

MacConkey's Agar Medium

This medium is both Selective and differential agar used for isolation of Gram-negative enteric bacilli and differentiation of lactose fermenters from non-lactose fermenters (Forbes *et al.,* 2007).

Eosin Methylene Blue Agar (EMB)

This medium is both a selective and differential agar. It inhibits Gram-positive bacteria, so it is instrumental in isolating Gram-negative bacteria. The normal flora bacterium *Escherichia coli* is readily detected on EMB agar by the green sheen that the colonies develop (McKane and Judi, 1996).

Mannitol Salt Agar

It is an indicator as well as selective medium. It contains Mannitol, NaCl (7.5%) and phenol red in nutrient agar. *Staphylococcus aureus* strains form colonies surrounded by yellow zones due to fermentation of Mannitol. NaCl inhibits the growth of other bacteria (Pachauri *et al.,* 2013).

Biochemical Tests:

Simmon's Citrate Agar:

Slants of Simmons citrate medium were inoculated with the test bacteria by stabbing, then tubes were incubated at 37°C for 24-48 hours. After incubation period the tubes were checked for Color change, blue Color indicated positive and green Colour indicated negative test (Dheyab *et al.,* 2018).

Catalase Test (Slide Test):

Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick. Place a drop of 3% Hydrogen peroxide (H_2O_2) on to the slide and mix. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling. A negative result is no bubbles or only a few scattered bubbles (Koneman *et al.,* 1997).

Oxidase Test:

Oxidase was detected by taking a filter paper soaked with the test reagent (tetramethyl-p-phenylenediamine dihydrochloride). Pick the colony to be tested with a wooden stick or loop and smear in the filter paper. Positive result observes inoculated area of paper for a color change to deep blue or purple within 10-30 sec. A negative result is no color change observed (Goldman and Green, 2015).

Coagulase Test:

This test used to detect coagulase, an enzyme produced by pathogenic *Staphylococci* (Vandepitte *et al.,* 2003).

Detection of Hemolysis on Blood Agar

All the isolates were tested for hemolysis after overnight incubation at 37 °C on blood agar. Hemolysis was recorded as alfa-hemolysis (α-hemolysis), beta-hemolysis (β-hemolysis) and Gama-hemolysis (γ-hemolysis) or (no hemolysis) *Staphylococcus aureus* ATCC 29213 used as positive control strain (Boerlin *et al.,* 2003).

Lactose Fermentation and Bile Salt Tolerance

It was done by streaking bacterial isolates on MacConkey agar (Deben Diagnostics Ltd, UK without crystal violet), then incubated at 37 ˚C for 24 hrs. The colonies that tolerate bile salt and ferment lactose appeared as pale pink to red (Irving *et al.,* 2004) .

VITEK-2 Compact System:

All the isolates were screened and identified through the VITEK-2 compact System (BioMérieux, Marcy L'Etoile, and France) at Nanakaly hospital Erbil - Kurdistan-Iraq, in accordance with the manufacturer's instructions. This is a phenotypic type of identification that depends on biochemical reactions to identify the isolates (Al-Hasan, 2013). Gram-Negative Card (GNC) is used for the automated identification of 135 taxa of the most significant non-fermenting and fermenting gram-negative bacilli. The GNC is based on established biochemical techniques and newly developed substrates measuring the source of carbon utilization, activities of enzymatic, and resistance. There are 48 biochemical exams and one negative control as well. While gram-positive Card (GPC) is used for the automated identification of 115 taxa of the most important non-spore-forming and primarily cocci. There are 43 biochemical tests measuring enzymatic activities, resistance and carbon source utilization. Final identification results were available in approximately eight hrs (Pincus, 2006).

Antimicrobial Susceptibility Testing (AST)

Susceptibility antimicrobial testing was performed for 18 different therapeutically relevant antibiotics by VITEK 2 compact System (AST - P592, AST - GN77 and AST- ST03) purchased from (BioMérieux, France). Minimum inhibitory concentration (MIC) was carried out in triplicate and the average MIC was calculated, as suggested by the Clinical and Laboratory Standards Institute

(CLSI). Separates resistant to three or more of antimicrobials classes were measured as multidrug-resistant isolates. The tested agents used five different of antimicrobials classes: the β-lactams (ampicillin 10 µg, amoxicillin/clavulanic acid 20 µg/10 µg , cefotaxime 30 µg, ceftazidime 30 µg, cefoxitin 30 µg, ceftriaxone 30 µg, cefepime 30 µg, cefazolin 30 µg, ampicillin/sulbactam 20 µg/10 µg, piperacillin/tazobactam 100 µg/10 µg, imipenem 10 µg, ertapenem 10 µg, and meropenem 10 µg), aminoglycosides (amikacin 30 µg, gentamicin 10 µg, and tobramycin 10 µg), FQs (ciprofloxacin 5 µg and levofloxacin 5 µg), antimetabolites (sulfonamides/trimethoprim 23.75 µg/1.25 µg), other antibiotic groups, Linezolid 30 µg and tetracycline 30 µg. The inhibition of zone was reported and measured as susceptible (S), sensitive (I), intermediate, (R), resistance according to the clinical and laboratory standard institute (CLSI) guideline (Patel *et al.*, 2015).

Statistical Analysis: Statistical Package for the Social Sciences (SPSS) and Microsoft Office Excel were used for data entry and analysis (Meidani *et al.*, 2013).

RESULTS

Socio-demographic status of cases enrolled in the study

This study was done on 70 cases (39 males and 31 females), male to female ratio was 1.25:1. The age mean was 41 years, ranging from (4 to 81) years. Nearly two-third of the cases resides in urban areas (62.9%) and one third (37.1%) were in the rural areas. The majority of cases were unemployed (75.7%), and (74.3%) of cases were married. Almost all of cases (94.3%) were inpatients, the educational level of the patients was nearly similar for illiterate, primary, secondary and high education (28.6%, 24.3%, 27.1% and 20% respectively), as shown in Table 1.

Table 1: Socio demographic status of cases enrolled in the study

Characteristics (n=70)		
Age group	**Frequency**	**Percent (%)**
<25	19	27.1
15-44	24	34.3
45-64	13	18.6
≥ 65	14	20.0
Gender		
Male	39	55.7
Female	31	44.3
Residency		
Urban	44	62.9
Rural	26	37.1
Employment		
Employed	17	24.3
Unemployed	53	75.7
Marital states		
Single	18	25.7
Married	52	74.3
Patient setting		
Inpatient	66	94.3
Outpatient	4	5.7

Education		
Illiterate	20	28.6
Primary	17	24.3
Secondary	19	27.1
High Education	14	20.0

Clinical parameter of the patients

Among examined patients 45(64%) of patients were febrile, and 63 (90%) out of 70 patients received chemotherapy. Most of the cases 50(71.4%) were non-smoking, a history of catheterization were positive in 28 (40 %) patients, as shown in Table 2.

Table 2: Clinical characteristics of the patients.

Clinical cases	**Frequency**	**Percent %**
Febrile		
Yes	45	64.3
No	25	35.7
Symptomatic		
Yes	32	45.7
No	38	54.3
Family history		
Yes	13	18.6
No	41	58.6
Not Sure	16	22.9
Started therapy		
Chemotherapy	63	90
Non chemotherapy	7	10
Smoking		
Yes	20	28.6
No	50	71.4
History of catheter use		
Yes	28	40
No	42	60

Distribution of Patients according to the types of hematological malignancies

Out of 70 cases of hematological malignancies 19 cases (27.1%) were diagnosed as acute lymphocytic leukemia (ALL), followed by acute myelocytic leukemia (AML) 17 (24.3%) and Non-

Hodgkin Lymphoma (NHL)12(17.1),chronic lymphocytic leukemia accounts for the minority with only one case 1 (1.4 %), as shown in Figure1.

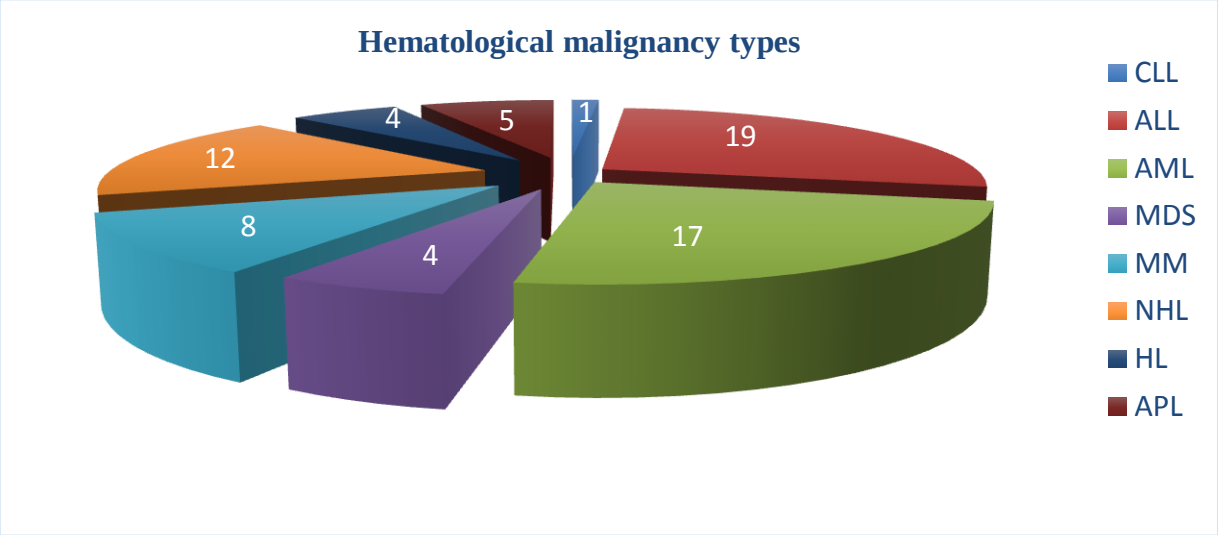

Figure 1: Distribution of types of hematological malignancies patients.

Bacterial profiles and site of isolation

Specimen culture was done for all 70 cases that are included in this study, bacterial pathogen was positive in 44 out of 70 patients, in some patient more than one specimen (different specimen) were send for culturing. Therefore growth had been detected in 56 out of 144 examined samples. Different type of specimens was taken from examined patients according to patients complain, among them bacterial growth could be easily detected from sputum samples which constituted 21(37.5%) of growths of all specimens followed by urine samples which constituted 17 (30.4%) of growths of all the specimens, blood samples came in the 3rd place which constituted 12(21.4%) followed by wound swab and throat swab, 3 (5.4%) of growths from all specimen samples. The predominant bacterial growth that had been isolated from the cultures of different specimens was *Escherichia coli* which accounts for 11(19.6%) of the isolates, followed by *Klebsiella pneumoniae spp pneumonia* and *Klebsiella pneumonia* which accounts for 6(10.7%) of the isolates for each of them. Fisher's Exact Test shows significant association between type of specimen and bacterial growth with a P value of <0.001. As shown in table 3 and 4.

Table 3: Result of culture according to specimen sites in patients with hematological malignancies

Culture	Blood	Urine	Sputum	Throat swab	Wound swab	Stool	Total
Growth	12 (21.4%)	17 (30.4%)	21 (37.5)	3 (5.4%)	3 (5.4%)	0 (0%)	56 (38.9%)
No growth	42 (47.7%)	30 (34.1%)	4 (4.5%)	1 (1.1%)	1 (1.1%)	10 (11.4%)	88 (61.1%)

Total	54 (37.5%)	47 (32.6%)	25 (17.4%)	4 (2.8%)	4 (2.8%)	10 (6.9%)	144 (100%)

Table 4: Distribution and predominate of bacterial isolates from different clinical specimens among hematological malignancies patients.

Isolated organism	Blood	Urine	Sputum	Throat swab	Wound swab	Total
Escherichia coli	3 (25%)	6 (35%)	0	1 (33.3%)	1(33.3%)	11(19.6%)
Klebsiella pneumoniae spp pneumonia	1 (8.3%)	2 (11.7%)	2 (9.5%)	0	1(33.3%)	6 (10.7%)
Klebsiella pneumoniae	0	1 (5.8%)	4 (19%)	1 (33.3%)	0	6 (10.7%)
Pseudomonas aeruginosa	0	0	1 (4.7%)	0	0	1 (1.7%)
Stenotrophomonas maltophilia	1 (8.3%)	0	0	0	0	1(1.7%)
Raoultella ornithinolytica	0	0	1 (4.7%)	0	0	1(1.7%)
Cedecea lapagei	1 (8.3%)	0	0	0	0	1(1.7%)
Sphingomonas paucimobilis	0	0	1 (4.7%)	0	0	1(1.7%)
Acinetobacter haemolyticus	2 (16.6%)	0	0	0	0	2 (3.57%)
Enterococcus faecalis	1 (8.3%)	2 (11.7%)	0	0	0	3(5.3%)
Streptococcus parasnguinis	0	0	4(19%)	1 (33.3%)	0	5 (8.9%)
Streptococcus viridans group except S. pneumoniae	0	0	1 (4.7%)	0	0	1(1.7%)
Staphylococcus aureus	0	1 (5.8%)	0	0	1(33.3%)	2(3.57%)
Staphylococcus haemolyticus	0	4 (23.5%)	1 (4.7%)	0	0	5(8.9%)
Staphylococcus saprophyticus	0	1 (5.8%)	0	0	0	1(1.7%)
Staphylococcus epidermidis	2 (16.6%)	0	0	0	0	2(4.0%)
Staphylococcus pseudintermedius	1 (8.3%)	0	0	0	0	1(1.7%)
Streptococcus pneumonia	0	0	2 (9.5%)	0	0	2(3.57%)
Streptococcus iniae	0	0	1 (4.7%)	0	0	1(1.7%)
Rothia mucilaginosa	0	0	1 (4.7%)	0	0	1(1.7%)
Streptococcus mitis/Streptococcus oralis	0	0	1 (4.7%)	0	0	1(1.7%)
Streptococcus viridans	0	0	1 (4.7%)	0	0	1(1.7%)
Total	**12(100%)**	**17(100%)**	**21(100%)**	**3(100%)**	**3(100%)**	**56(100%)**

Gram stain isolation according to different site of infection

Based on gram staining, gram-negative bacteria (GNB) were higher than the growth for gram- positive bacteria (GPB) [30 (53.6%) vs. 26

(46.4%)]. Gram-negative bacterial growth from samples taken from blood, throat and wound swabs were (66.7%), followed by urine culture (52.9%) and sputum cultures (42.9%). On the other hand gram-positive bacteria growth from samples taken from sputum accounted for (57.1%), followed by urine cultures 8(47.1%) while blood, throat swabs and wound swabs came in the 3rd stage which accounted for (33.3%) of each of them, Chi-square test shows no statistical significant association between the gram stain and specimen sites as shown in Table 5.

Table 5: Association between grams stain and culture specimen sites (Column percent).

Gram stain	Blood	Urine	Sputum	Throat swab	Wound swab	Total
Gram Negative	8 (66.7%)	9 (52.9%)	9 (42.9%)	2 (66.7%)	2 (66.7%)	30 (53.6%)
Gram Positive	4 (33.3%)	8 (47.1%)	12 (57.1%)	1 (33.3%)	1 (33.3%)	26 (46.4%)
Total	12 (100%)	17 (100%)	21 (100%)	3 (100%)	3 (100%)	56 (100%)

Prevalence of bacterial infections among hematological malignancy patients

The prevalence of infection was highest among patients with acute leukemia as ALL accounts for (16) cases, AML (8), APL (3), NHL (7). The least growth of bacteria was found among patient with MM witch counts for (3), followed by MDS (3), and HL (3). Among patients included in this study only one patient was diagnosed as chronic lymphocytic leukemia CLL. Fissures exact test shows no significant association between types of hematological malignancies and bacterial growth, as shown in Figure 2.

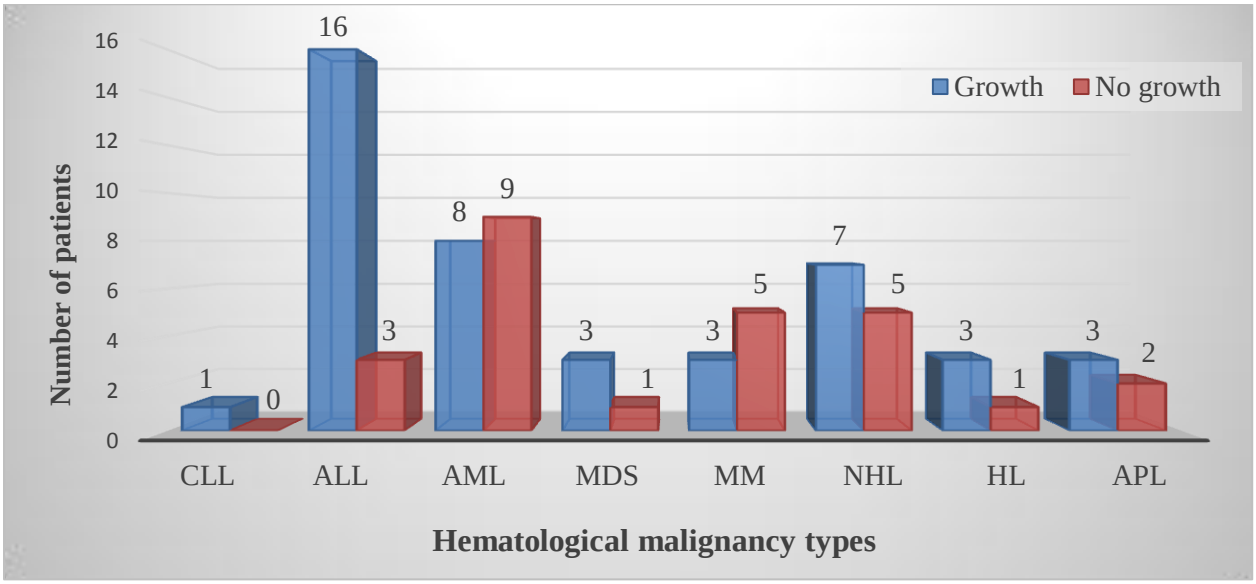

Figure 2: Distribution of bacterial infections among hematological malignancy patients.

Antibiotic susceptibility test (AST) to GNB and GPB isolates

Antibiotic sensitivity test and gram staining performed for all patients included in this study, showing that GNB were highly resistance to ciprofloxacin, gentamycin, sulfamethoxazole /trimethoprim, piperacillin and cefepime, but sensitive to imipenem, amikacin and tigecycline. While GPB highly resistance were to tetracycline, ceftriaxone, levofloxacin, ampicillin and erythromycin, but highly sensitive to linezolid, Tigecycline, Imipenem and vancomycin, as shown in Table 6.

Table 6: The percentage of susceptibility pattern of Gram-negative and Gram-positive bacteria to 18 various antibiotics using VITEK-2 compact system.

Isolated bacteria	**Gram-negative bacteria**			**Gram-positive bacteria**		
Antibiotic scientific name	MIC Interpretive Criteria (µg/mL)			MIC Interpretive Criteria (µg/mL)		
	R	S	I	R	S	I
Ciprofloxacin	60.0	10.0	10.0	0	9.7	3.2
Gentamycin	46.7	30.0	10.0	18.8	18.8	43.8
Tobramycin	30.0	26.7	3.3	25.0	25.0	6.3
Amikacin	10.0	46.7	0	x	6.3	x
Piperacillin	35.5	12.9	3.2	4.5	4.5	x
Amoxiclav/ clavulanic acid	25.8	12.9	12.9	4.5	9.1	x
Cefepime	32.3	16.1	6.5	x	x	x
Ampicillin	29.0	x	x	52.9	x	x
Imipenem	16.6	79.0	x	x	75.0	x
Erythromycin	29.4	x	x	42.3	11.5	7.7
vancomycin	17.4	4.4	x	3.3	63.3	x
Tetracyclines	33.3	4.2	4.2	72.0	12.0	4.0
Sulfamethoxazole /Trimethoprim	39.3	25.0	3.6	33.3	20.0	x
Nitrofurantion	6.7	16.7	x	x	38.7	3.2
ceftriaxone	25.8	12.9	3.2	58.8	5.88	x
Levofloxacin	26.7	3.3	10.0	53.8	6.5	6.5
linezolid	x	10.7	x	4.1	79.0	x
Tigecycline	3.6	39.3	x	x	75.0	x

S: Sensitive, R: resistant, I: Intermediate, X: Not performed.

DISCUSSION

Cancer is one of the major global health problems worldwide. Patients with hematological malignancies are extremely susceptible to nearly any kind of bacterial infection .The overall prevalence of bacterial infection among hematological malignant patients were 44 (62.9%) out of 70 cases, which differ with the result from study done in Iran, reported that the prevalence of infection among cancer was (24.6%) (Eslami Nejad *et al.,* 2010), and with the study done in Romania revealed that the frequency of infection were (14.92%) (Adipocytes, 2014). Our result close to previous study done in Iraq and Sudan showing the frequency of infection among cancer patients were (mean of 44.2%), and (48%) respectively (Almaziny, 2014, Nurain *et al.,* 2015). The difference in prevalence might be explained by the fact that patients profile, environmental and geographical factor play a major role in such group of patients (Grasgruber *et al.,* 2018).

The hematological malignancies (HMs) were slightly higher among male gender (39 males and 31 females) the male: female ratio in our study was 1.25:1. Our study was in agreement with other study done in Iran, that the incidence of cancer was higher in men than women with the ratio of (1.25). The maximum gender ratio was reported in Hamadan (1.45) and the minimum was reported in Yazd (1.15) (Mashhadi *et al.,* 2010). Other studies also showing male predominance among hematological malignant patient, such results lacks a clear explanation but occupation exposure , hormonal influence and migrate smoking might have a role, further studies are warranted both experimentally epidemiological for further explanation such association (Kim *et al.,* 2018). In our study majority of cases (71.4%) were non-smoker, however many studies show association of smoking with hematological malignancy(Brownson *et al.,* 1993), while others shows no association(Kasim *et al.,* 2005, Ugai *et al.,* 2017). Further studies are reasonable to further investigate such association.

Among homologically malignant patients included in this study, the prevalence of infections was more common among patients with acute leukemia (38.5%). This result was similar to those studies that have been done in India and in China showing similar infection prevalence among all patients (46.5%), (34.3%) respectively (Gupta *et al.,* 2019, Tang *et al.,* 2020). Gram-negative bacteria were higher than gram-positive bacteria. Several studies in recent years have noted a shift of prevalence from GPB to GNB (Gudiol *et al.,* 2013, Kokkayil *et al.,* 2018). A study from Italy, consistent with this study reported that Gram-negative bacteria were isolated as the most common cause in patients with hematological malignancies than Gram-positive bacteria. Several studies reported that, GPB was the main cause of bacterial infection as compared to GNB. In hematological neoplasia (Eslami Nejad *et al.,* 2010, Fentie *et al.,* 2018a). This finding incompatibility with this study.

The bacterial isolates detected in our study showing that Gram-negative bacteria were higher than gram-positive bacteria. This result is in accordance with other studies done in Italy, reporting that Gram-negative bacterial isolated were the most common type in patients with hematological malignancies than Gram-positive bacteria (Eslami Nejad *et al.,* 2010, Fentie *et al.,* 2018a). Several other studies in recent years have noted a shift of prevalence from GPB to GNB (Gudiol *et al.,* 2013, Kokkayil *et al.,* 2018). The predominant bacterial isolates from our study was *Escherichia coli* which constituted (19.6%) of the isolates, followed by *Klebsiella pneumoniae spp pneumonia* and *Klebsiella pneumonia* which constituted (10.7%) of the isolates for each of them, similar results were found in Tehran-Iran that illustrated as *Escherichia coli* was the most common microorganism isolated from the leukemia patient (Abdollahi *et al.,* 2016). Several other study at international level had similar result, showing that the bacterium *E. coli* was the most prevalent gram-negative isolated bacteria (Alcala-Guanzon and Tan-Torres, 1998, Ashour and El-Sharif, 2009). Furthermore, in this study *E. coli* was among predominant GNB isolated from patient with septicemia, which accounts for 8 of 12 isolates (66.7%). A study done in India showing a similar result (Mathur *et al.,* 2002); (Ghosh *et al.,* 2012).

A study from Germany illustrated that the most prevalent gram-positive bacteria in respiratory tract infections (RTIs) in cancer patients were *Streptococcus* and *Staphylococcus species* while *Klebsiella pneumonia* was the common isolated gram-negative bacteria from throat and sputum (Hoheisel *et al.,* 2003). In compatibility with this study our result showing

that 11[21(52.3%)] isolated GPB from RTIs were *Staphylococcus* and *Streptococcus species* whereas among GNB, *Klebsiella species* accounts about 4 [21(19%)] from sputum followed by 1[3(33.3%)] from throat swab.

In current study *E.coli* 6[17(35 %)] was the most common bacterial isolate among patients with UTI, followed by *K. pneumoniae* 3[17(17.6 %)]. While the predominant species among gram-positive isolates were, *Staphylococcus haemolyticus* 4[17(23.5 %)], followed by *Enterococcus faecalis* 2[17(11.7 %)]. This is in accordance with other studies showing that Gram- negative bacteria are highly associates with UTI patient (Kline and Lewis, 2017).*Escherichia Coli* is the most frequently isolated bacteria from the urine, blood, throat, and wound swab cultures in our study, since it is the most prevalent commensal inhabitant of the gastrointestinal tract, it is a common pathogen liked with community-associated as well as hospital-acquired infections (Drago *et al.,* 2010). Several other studies confirming similar results(Ashour and El-Sharif, 2009;Nurain *et al.*, 2015) (Chiu and Chang, 2009, Sharma *et al.*, 2011).

Regarding antimicrobial resistant pattern, our study showing that GNB were highly resistant to Ciprofloxacin, Gentamycin, Sulfamethoxazole /Trimethoprim, Piperacillin, and Cefepime, whereas they are highly sensitive to Imipenem, Amikacin and Tigecycline. While GPB showing highly resistant for Tetracyclines, ceftriaxone, Levofloxacin, Ampicillin and Erythromycin, but highly sensitive for linezolid, Tigecycline, Imipenem, and vancomycin.

Comparable with this result, several other studies reported that, GNB were highly resistant against cephalosporins, quinolones and penicillins (Marin *et al.,* 2014, Yao *et al.,* 2017). Another study demonstrated that antibiotics resistance among GPB were more common for tetracycline's, penicillins, erythromycin and quinolones (El Haddad *et al.,* 2018). Furthermore, in compatibility with our result, a retrospective study in Iraqi showed that the susceptibility of gram-positive organisms to antibiotics Imipenem was found to be the most effective, as for gram-negative organisms, demonstrated the highest sensitivity to Amikacin (Al-Zubaidy *et al.,* 2020). On the other hand, a study from Malaysia demonstrated that all isolated gram-negative bacteria were resistant for Ampicillin, cefazolin, ceftriaxone, piperacillin. Whereas all isolated gram-negative bacteria were extremely resistant to imipenem which disagree with our finding (Baskaran *et al.,* 2007).

Today development of MDR is become natural phenomenon, due to interestingly raise in the number of immunocompromised conditions, blind and improper use of broad spectrum of antibiotics as well as poor infection prevention, beside that patients profile, environmental and geographical factor were among important player determining the bacterial profile and resistance pattern (Weinstein, 2001); (Lebea and Davies, 2017, Weinstein, 2001).

CONCLUSION

Acute leukemia was the common hematological malignancies with slight male predominance. Gram negative bacteria isolates were higher than gram positive bacteria. *Escherichia coli* was the most frequently isolated bacteria. The majority of bacterial isolates were resistant to various antibiotics. Among GNB were highly resistant to Ciprofloxacin, Gentamycin, Sulfamethoxazole /Trimethoprim, Piperacillin, and Cefepime, Also highly sensitive to Imipenem, Amikacin and Tigecycline. While among GPB highly resistant was to Tetracyclines, ceftriaxone, Levofloxacin, Ampicillin and Erythromycin, Also highly sensitive to linezolid, Tigecycline, Imipenem, and vancomycin

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CONFLICTS OF INTEREST

There are no conflicts of interests to be declared.

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RESEARCH PAPER

Liver Molecular-biochemical Markers in Viral Hepatitis B patients

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A B S T R A C T:

The prevalence of liver disease has led to its emergence as a major challenge. This challenge is also attributed to factors such as the challenging diagnosis, complexity of pathogenesis, and the absence of established therapies. When Hepatitis B virus (HBV) infections occurs in patients that do not have detectable hepatitis B surface antigen (HBsAg), it is referred to as occult infections. Despite the fact that these kinds of infections have been found in patients with chronic hepatitis C liver disease, there is still no evidence on their clinical implication and prevalence. HBV is a small partial deoxy ribonucleic acid (DNA) virus with like retroviruses. The virus falls under the group of Hepadnavirus infections and family of orthohepadna virus. About 66% of patients with acute HBV infection have an asymptomatic, mild, and sub-clinical illness that typically goes undetected. Thus, in this work, hematological parameters were used in detecting the virus, and the results of the hematological parameters revealed significant changes in white blood cell (WBC), lymphocyte and platelet area under curve (AUC) (0.95), (0.66), and (0.82).
The mean values for ALT and ALP in HBV-positive patients increased significantly as compared to the control. Similarly, there was no significant difference between the AUC, CI for HBV-positivity for ALT 0.91, (0.8485-0.9854) and ALP 0.89 (0.8123-0.9633). This study revealed a significant positive correlation between ALP level and ALT and each lymphocyte, granulocyte and WBCs. Thus, each of them may be considered as major factors of development of HBV level.

KEY WORDS: HBV, qPCR, Liver test.
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INTRODUCTION:

Even with the availability of HBV vaccines and treatment drugs, it is still regarded as a major global health challenge, with over 350 million people having chronic infections worldwide, and almost 1 million people die from the complications associated with HBV [1]. The liver can be damaged to different extents as a result of persistent HBV infection, and this in turn results in cirrhosis, hepatitis, hepatocellular carcinoma (HCC), and fibrosis [7]. HBV belongs to the family of noncytopathic hepatic DNA virus, which can only infect the liver cells of orangutans and humans, and the possibility of HBV to infect other cells that are non-hepatocyte is yet to be proven [8].

Hepatocyte lesions are not directly caused by HBV infection, ways the host's immune responds to the infection determines it clears the virus of causes liver disease. While, transient and self-limiting hepatitis is developed in HBV-infected adults, and 95% of infections end with the elimination of the virus and establishment of protective antibodies, a large number of neonatal vertical transmission of HBV from mother to child leads to chronic infection [9]. Regardless of the fact that there are many factors that contribute to the pathogenesis of the virus, persistent and chronic HBV infection is a complex process which involves the interaction of the virus and the host immune system, thereby incapacitating the adaptive and innate immune response [8]. Chronic hepatitis B virus (HBV) is a frequently occurring human viral infection that affects millions of individuals globally, and is a major cause of morbidity and mortality [10]. Hepatitis B virus is a small partial deoxy ribonucleic acid (DNA) virus with like retroviruses [11]. The virus falls under the group

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of Hepadnavirus infections and family orthohepadna virus. About 66% of patients that are chronically infected with HBV infection have asymptomatic, slight, and sub-clinical illness that typically goes undetected. The disease has a compound history, where its development involves various stages or phases. More active liver disease and high level of HBV DNA in their serum are known to be predisposing factors to the development of cirrhosis and other liver diseases. These are the people who are mostly recommended for treatment.

Patients with Hepatitis will be easily identified due to their elevated serum aminotransferase level. More so, their liver biopsy findings will show a reading with stage 1-3 fibrosis and grade 1-3 inflammation. To some extent, patients with cirrhosis will quickly be pointed out, due the presence of chronic liver infection and a reduced number of platelet counts. The presence of the hepatitis B markers now makes the identification of patients with liver complications advanced to a greater extent. The Agency for Healthcare Research and Quality on the administration of drugs has continuously reported that patients at various clinical phases of Complete Heart Block (CHB) have been undeniably described since antiviral treatment guides for hepatitis B rely upon explicit classes of patients [12].

There is an interaction between HBV and receptors like TLR2/4 that are manifested on Kupffer cells for the production of a large number of inflammatory cytokines and chemokines (TNF, CCL2), which in turn cause damage to the liver [13,14]. By means of the inflammatory mediators, the peripheral monocytes are induced to permeate the liver, and then proliferate and differentiate into macrophages. This in turn, intensifies the production of inflammatory chemokines and cytokines, further inducing the development of liver inflammation and fibrosis [15,16]. Furthermore, immunomodulatory molecules (IL-10, TGF-β, PD-L1/2) which inhibit the anti-fibrotic T cells and NK cells, are produced by HBV-induced suppressive monocytes/macrophage; the produced immunomodulatory molecules in turn secrete cytokines like PDGF and TGF-β so that the HSC can be activated, thereby allowing the survival of HSC [17,18]. In recent times, it has been reported that patients with HCC and cirrhosis exhibit increase in the numbers of ascetic and peripheral MDSC, but its effect on such pathology was not established [19]. Theoretically, the function of the NK cells and T cells are impaired by HBV-induced MDSCs which also inhibit the IFN-γ secretion through CTLA-4 or PD-L1 [20]. Similarly, the cytotoxicity of NK cells are inhibited by the MDSC through TIGIT or PD-L1 [21,22], thereby preventing the HSC from being killed by the NK cell.

In this paper, the recurrence of these markers has been determined in various clinical phases of chronic hepatitis B infection as portrayed in this paper. The study participants were grouped into two groups, one consisting of normal (control group) and the other group consisting of hepatitis B patients (HBV positive). The control group (n = 40) had 20 males and 20 females, while the hepatitis B virus positive group (n = 40) had 25 males and 15 females.

Liver Anatomy

The liver is the largest organ of the human body with a weight of around 1500 g, and is positioned in the upper right corner of the stomach. The organ is intently connected with the small digestive tract, setting up the supplement rich venous blood that leaves the body-stomach related tract. The liver performs over 500 metabolic tasks, achieving the synthesis of items that are released into the circulation system (for instance glucose got from glycogenesis, plasma proteins, coagulating parts and urea), or those that are discharged to the extensive intestinal tract (bile). In addition, the liver parenchyma serves as a storage for numerous items such as glycogen, fat- and fat-solvent supplements) [23].

A good number of researchers have attributed the hepatocellular damage resulting from chronic hepatitis B infection (CHB) to increase in oxidative stress [1-4]. Nevertheless, in majority of these works, only the individual antioxidants measurements of patients have been used for the evaluation of oxidative status, and there is limited information about the total antioxidant response (TAR) of subjects with cirrhosis and CHB caused by hepatitis B virus (HBV) [5]. Based on the review of literature done in this study, the literature is lacking in information about oxidants in subjects with cirrhosis resulting from HBV infection, as well as information on antioxidants and oxidants in inactive hepatitis B carriers.

Liver Disease

One of the significant roles played by the liver is to process all ingested food substances. It can either convert the elements into a useful product or waste products which are eliminated at the end of the process [24]. The liver, just like other organs that comprise a complete human body, can be damaged in or inflamed as a result of abnormal conditions. Some of the complications associated with the liver are discussed below [25].

Hepatic steatosis is considered to be the most common liver disease, and it may be reversed. It often affects individuals who have Crohn's sickness, and ulcerative colitis. In this complication, fats become deposited in the liver affecting the accommodation of liver cells, hence affecting the performance and functioning of the liver in general. Such complications are likely to affect individuals with diabetes, obesity, and those who are pregnant as well. Individuals using steroids also stand a chance of suffering from the same complications. In most cases, these conditions do not require to be corrected as it bears minimal effects [24].

Another liver disease is Gallstone; the condition continues to affect individuals with Crohn's illness. The situation leads to cases whereby the bile is withheld until it is required for absorption. In such a fact, bile tends to become hard leading to the formation of stones in the gallbladder. Such a case leads to the blockage of the bile duct as a result of the need for the rocks to leave the gallbladder. Such an issue may, however, be resolved by the treatment of the gallbladder [26]. The bile channel may be affected through aggravation by primary sclerosing cholangitis (PSC). Such a condition often tends to change in patients who have ulcerative colitis, but rarely in those who have Crohn's malady. A small number of patients experiencing PSC tend to lack IBD. Complications of this kind leads to irregularities in the bile stream. The damage of the bile may lead to the development of jaundice and tingling. Such a condition is often difficult to control. Stents may be used in the control of bile development in order to enhance the bile streaming process [24]. Primary Sclerosing Cholangitis entanglement may cause bile contamination and excessive growth of bile channel. In such a case, the liver becomes excessively damaged, and transplantation may be required. Colon malignant growth is yet another complication likely to affect patients with PSC. In such a case, patients are required to seek regular checkups [27].

Liver cancer caused by HBV

Hepatitis B virus is the most common factor leading to liver cancer. Hepatitis virus link is associated with liver cancer in the sense that it causes an inflammation of the liver, and over time after it has become chronic hepatitis, it stops the liver from carrying out its important duties such as filtering toxins and controlling blood glucose. Out of the nine cancers, hepatocellular carcinoma is the one majorly caused by Hepatitis C and B viruses. Worldwide, 54% of people infected with liver cancer have Hepatitis B. Extra hazard factors for developing liver cancer growth include cirrhosis and smoking, exorbitant alcohol use, just as diabetes and obesity. Some inherited ailments that cause liver harm, also increase the possibility of developing liver cancer. Race or ethnicity and a family history of liver cancer growth are regarded as risk factors. Liver cancer growth is more typical among men than women, regardless of factors such as race or ethnicity [28].

Hepatitis B Virus

The HBV is a small partial DNA virus with like retroviruses [11]. Hepatitis B virus falls under the group of Hepadnavirus infections and family of orthohepadna virus. About 66% of patients with acute HBV infection have asymptomatic, mild, asymptomatic and subclinical illness that typically goes undetected. HBV is a DNA-containing virus. However, the replication occurs by means of an RNA middle of the road, a feature that places hepadna viruses near retroviruses. The remarkable features of the HBV replication cycle show the ability of the virus to survive in infected cells. This virus has an impact on the liver, making some parts of the body not to function normally. In the case of constant hepatitis, liver cirrhosis. The infection spreads through the blood and body liquids, making it simple to spread [29].

Molecular structure of HBV

HBV is a small, partly double stranded DNA virus, enveloped, and hepatotropic virus that is spherical with filamentous structures and relaxed circular DNA genome [28].

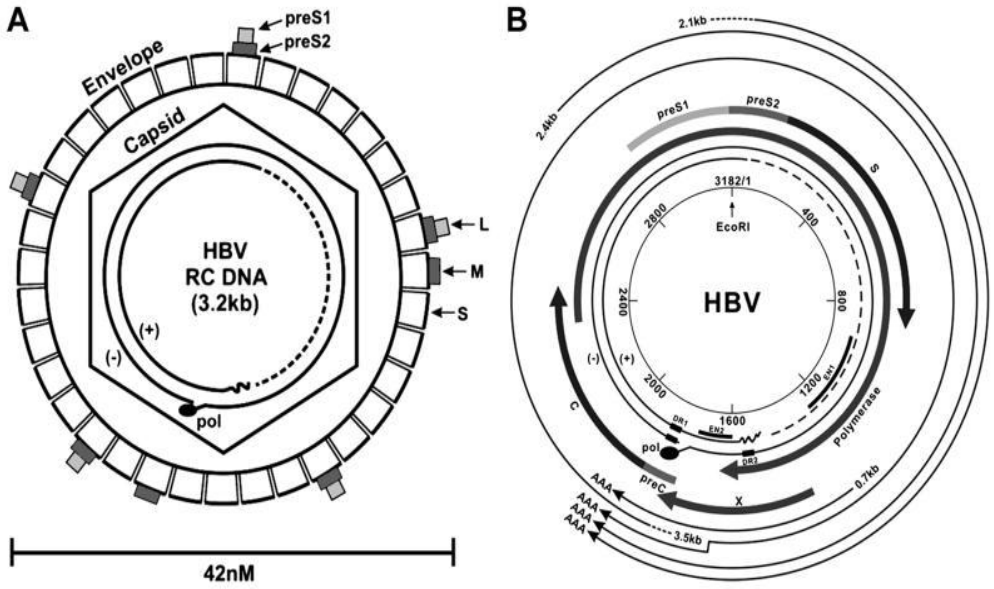

Figure 1: Molecular structure of HBV (Ghany M *et al.,* 2007)

Mostly, the HBV genome contained within the capsid appears in form of a double stranded and fragmented hover, with the polymerase protein combined with the "minus" strand in a covalent manner (figure1 A). the circular map of the HBV genome alongside nucleotides numbered from the single EcoR1 site (by tradition), transcripts alongside their polypeptide products. It is regarded as unique because it consists of DNA that is not twofold stranded (figure1 B). [30].

Mode of transmission and prevention

The most common mode of transmission is perinatal, whereby an infected mother passes on the virus to the child at birth. Another common mode of transmission is horizontal transmission, which is a transmission whereby the virus is transmitted to an uninfected person through the blood of an infected person, e.g. through accidental needle pricks. Another way is through sexual contact with an infected person, and in this mode a person gets infected through contact with the body fluid of the infected person. Therefore, efforts such as education and vaccines must be directed towards the avoidance of high-risk behaviors. Also, in several countries where the prevalence of HBV is low, there are infant vaccination programs and adolescents are also vaccinated against HBV so that they do not get infected even if engaged in sexual behaviours. Researchers have suggested that people in high risk areas should be vaccinated, and people who have been exposed to the virus not after two weeks of the prophylaxis therapy [31].

HBV genotypes and vaccines

Currently, there are ten known types of genotypes, and these also have mutants (A-J). They are classified according to geographical distribution as well as their response to antiretroviral therapy. The different genotypes contribute to the different characteristics in people with HBV worldwide as each their own characteristic. Recombinant hepatitis B immunizations are of the A2 genotype, which is one of the ten known genotypes whose circulation shifts internationally. Reports of uncommon HBV contaminations in blood donor with an imbalance of non-A2 genotype HBV in vaccinated subjects have brought up issues about the cross-insurance managed by HBV-A2 antibodies. Contaminations in HBV vaccines were asymptomatic and transient, demonstrating that immunization could result in clinical infection. Preclinical data demonstrates cross-reactivity and cross-assurance

by A2 antibodies against non-A2 HBV genotypes. Significant enhancements in HBV control have been exhibited in nations with differing genotype dispersion that have presented general youth HBV immunization programs. Available information shows that current HBV-A2 vaccination are of great importance in the prevention of infections and clinical disease caused by all the known HBV genotypes [32].

Materials and Methods

Participants

The study recruited n= 80 cases with a mean age of 47 years participants were grouped into two groups, (normal control) and viral Hepatitis B (HBV-positive). The control group (n=40) had 20 males and 20 females, while HBV-positive group (n=40) had 25 males and 15 females.

The study was conducted at the public health laboratory of Erbil city / Kurdistan region /Iraq from the month of October 2018 to the month of February 2019.

Sample Collection

The blood was collected from arm vein by using sterile disposable syringe. This, however, posed a danger because of the rupturing of the blood vessels. This means that, only sterile materials were used in this process so as to reduce the emergence of other health risks associated with puncturing of blood vessels such as spreading of germs and infections. Blood from the elbow bend, forearm and the back of the hand was used as the sample in the public health laboratory Erbil. The blood was extracted with standard specimen collection jell tubes. About 6ml of blood treated with EDTA was used to as the sample. The purpose of treating the blood with EDTA was to prevent the blood sample from clotting. The blood from veins was used because it contained all the wastes and diseases as it moved around the body, and it had not been purified in the liver, thereby making the concentration of the virus in the venous blood high. After the blood samples were taken, the centrifuge was fixed at the rpm of about 4000 because the serum was being worked on.

DNA Extraction

The EZ1 automated machine ,simultaneous purification of viral nucleic acids from serum samples using artus® HBV RG PCR kits were used in this work, and their contents are Elution tubes (1.5ml), QIAGEN® protease (lyophilized), Protease re -suspension buffer 6 ml, Carrier RNA 310 µg, Buffer AVE 2ml with adding 2ml of each samples.

Real Time PCR

Real time PCR serves as a method of monitoring how the polymerase chain reaction progresses so that the replicas of the DNA region of interest can be generated (in this case, it is the DNA of Hepatitis B). With the use of this technique, the amount of Hepatitis B virus was determined. There are three main steps involved in the process, and they are given as follows:

Denaturing (95 ^{O}C for 15 secs): here, the DNA's double stranded template was heated so that it can be separated into two single strands. Annealing (55 ^{O}C for 30 secs): this involved lowering of temperature to enable the DNA primers to initiate the attachment to the DNA template.Extending (72^{O}C for 15 secs) the temperature was again increased so as to make the new strand of DNA using the Taq polymerase enzyme, These three stages were repeated about 40 times so that the yield of the replicated DNA can be increased. Electrophoresis was then employed after the three processes were repeated so as to determine the size and quantity of the DNA fragment produced.

By means of the Real-time PCR, the amplification of PCR and location are consolidated into a solitary step. This highlights the relevance of using gel electrophoresis to detect items, and ultimately, it makes the strategy to be quantitative. With real-time PCR, the use of fluorescent dyes is employed in the labeling of PCR products during the process of thermal cycling. More so, using the real-time PCR instruments, the collection of fluorescent signals were measured during the exponential period of the reaction. This was aimed at achieving rapid and accurate evaluation of PCR products as well as objective data analysis by Rotor gene Q.

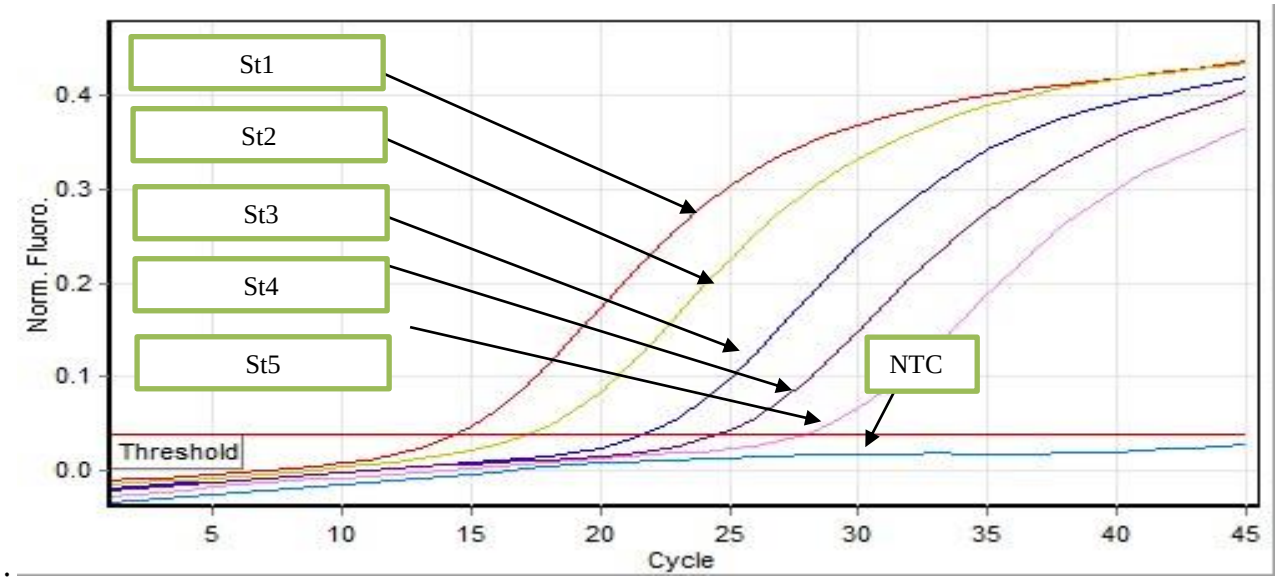

Figure 2: Five standard curve for quantitation detection of HBV standards No Template Control (NTC)

Data Analysis:

One-way ANOVA t-test was used for liver test parameter with chi-square . A P-value less than 0.05 was considered statistically significant. For PCR data interpreting using the software provided with the Rotor-Gene Q Series Software 2.1.0 this algorithm allows the direct comparison of the samples that have different starting fluorescence levels with the quantitation curves of samples. The Statistical Package for the Social Sciences program was used for data analysis GraphPad Prism 8.

Results

Liver function parameters

In the multiple regression analysis performed in this study, ALT ($p<0.0001$) and ALP ($p<0.0001$) were identified as independent predictors of HBV ($r2 =0.67$, $p>0.001$). The mean values for ALT and ALP in HBV-`positive patients increased significantly as compared to that of the control group. Similarly, there was no significant difference between the AUC (CI) for HBV-positivity for ALT 0.91 (0.8485-0.9854) and ALP 0.89 (0.8123-0.9633). On the other hand, total bilirubin, serum albumin and AST were not significantly associated with the HBV-positivity even after excluding AST from the model since it is associated with ALT.

Sensitivity test revealed that HBV- positivity did not affect the accuracy of AST, total bilirubin and serum albumin, However, their (Mean± SE) was found to be lower in HBV positive patients, normal control : AST 31.54 ± 1.63 vs 29.14 ± 1.44 $p=0.21$, Total bilirubin 0.69 ±0.09 vs 0.44 ± 0.03 $p=0.063$, and serum albumin 3.75 ± 1.63 vs 3.72 ± 0.07), but were not significantly ($p=0.073$) different from the control group.

Table 1: Linear Regression analysis for Viral load as dependent variable with ALT, AST, ALP, as independent Variables.

Parameters	AUC	95% CI	P value	Mean ± SE	
				Control	HBV+ve
ALT (UI/L)	0.91	0.8485 to 0.9854	<0.0001	17.48 ±1.375	34.19± 1.604
AST (UI/L)	0.60	0.4591 to 0.7394	ns	31.54± 1.625	29.14± 1.441
ALP (UI/L)	0.89	0.8123 to 0.9633	<0.0001	280.2± 14.08	502.2± 29.63
Total bilirubin (mg/dL)	0.71	0.5857 to 0.8488	ns	0.6913± 0.06060	0.4454± 0.03213
Serum Albumin ()	0.51	0.3727 to 0.6424	ns	3.755± 0.09355	3.720± 0.07393

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase , NS: not significant

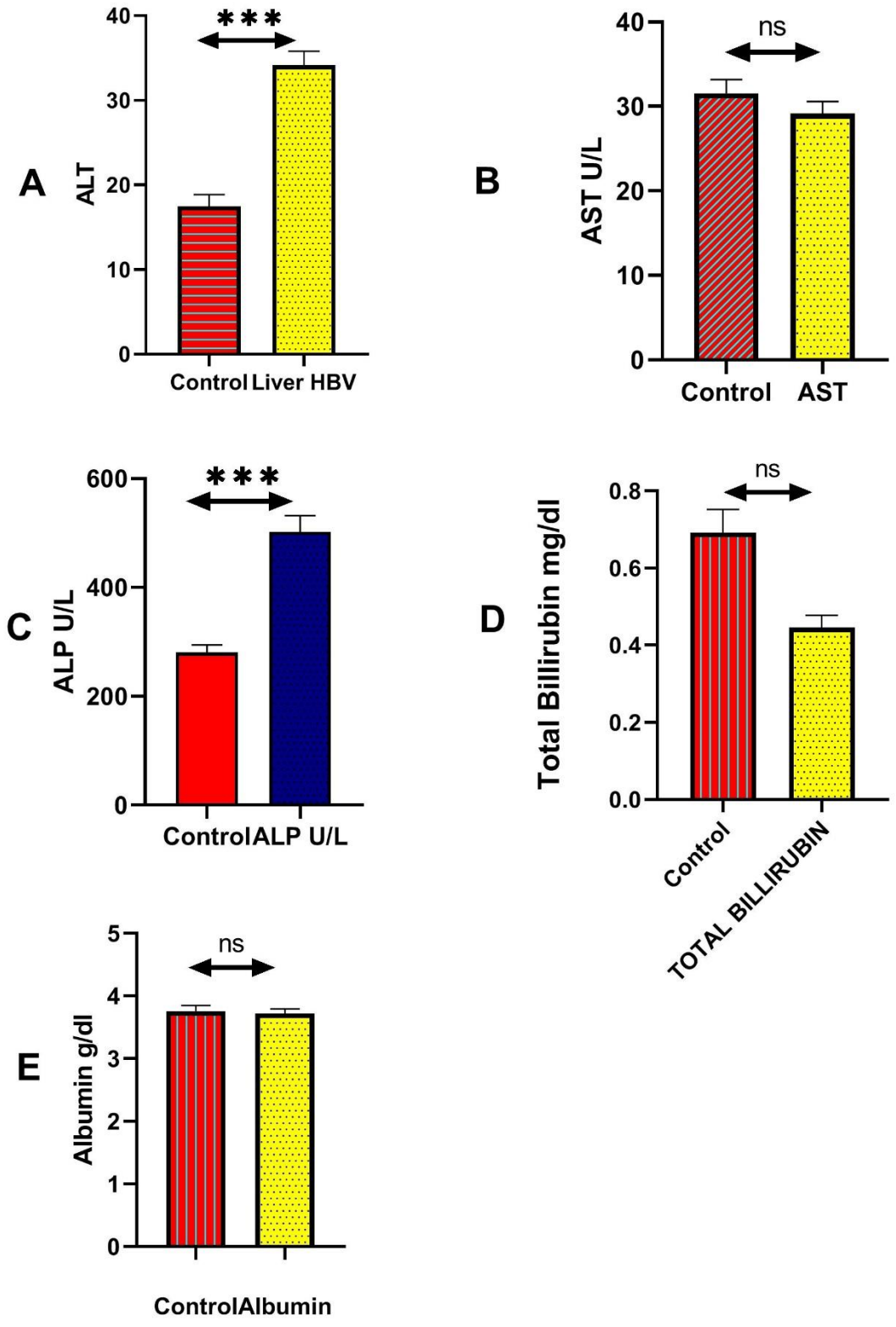

Figure 3: Box plot show comparison between control and HBV patient for liver tests parameters: (A) ALT: Alanine transaminase, (B) AST: Aspartate transaminase,(C) ALP: Alkaline phosphatase, (D) Total bilirubin. (E) Serum albumin

Correlation between liver function test and hepatitis B virus.

Linear regression analysis was used to determine the correlation between liver function levels (AST, ALT, ALP, total bilirubin, serum albumin) and hepatitis B viruses among the patients of the study samples, no correlation was found between ALT and HBV (-0.1509, p= ns), but a positive correlation was found between AST and HBV (0.0018), whereas it can be seen from table (4) which shows the liver function and hepatitis (ALP, serum albumin), there was no correlation between them except total bilirubin .

Table 2: Linear regression analysis for viral load as dependent variable with alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, albumin as independent variables.

Stat. Variables / Liver Function tests	R	95% CI	P value
ALT	-0.1509	-0.4452 to 0.1729	ns
AST	0.001875	-0.3223 to 0.3257	ns
ALP	-0.09158	-0.3919 to 0.2264	ns
Total bilirubin	0.06964	-0.2650 to 0.3893	ns
Serum Albumin	-0.3014	-0.5665 to 0.02023	ns
ALT	-0.1509	-0.4452 to 0.1729	ns

In order to obtain the CT value, quantitation information standard curve was run on software version 2.1.0.9. The CT value (CT = -3.287*log (conc) + 34.370) refers to the cycle number at the point that the amplification curve goes beyond a threshold (0.06182) of detection. In order to determine the CT value for each of the curves, a threshold line is set and the intersection between the curves is calculated. R2 value (correlation coefficient): the R square value (0.99971), or R2 value refers to the percentage of data that corresponds with the hypothesis that the standards form a standard curve. In an event of low R2 value, then it is difficult for standards to fit into a line of best fit. This may have a bearing on the reliability of the results, which are the calculated concentrations. A good R2 value is approximately 0.99. M and B. the calculations of the slope (M) and the intercept (B) of the standard curve are done automatically through the use of the formula y = Mx + B, and shown in the “Standard Curve” Reaction. Efficiency Threshold: The “Reaction Efficiency Threshold” refers to an alternate approach that is used in eliminating noise from the analysis. In this normalizing algorithm, the reaction efficiency estimation techniques are employed in comparative quantitation. Any sample that lacks a reaction efficiency of at least this level are excluded and a flag “NEG (R.Eff)” will be shown in the “CT Comment” column. A level of 0% shows that at the exponential stage, there was no reaction. On the other hand, 100% is indicative that a totally efficient reaction occurred at the exponential phase. In addition, when the percentages are negative, it means that there was a decline in the signal during the exponential phase.

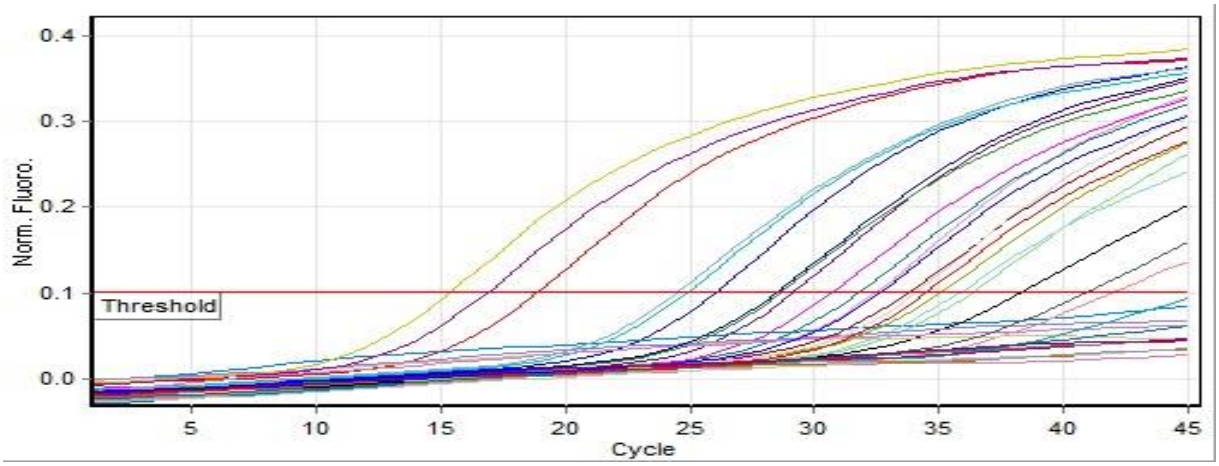

Figure 3: Quantitation data of Q PCR detection of hepatitis HBV virus

Discussion

This study evaluated a cohort of 80 participants out of which 40 were patients on various stages of viral hepatitis B infection. More so, 40 of them were the normal case with no detectable HBV by PCR. All the infected patients had detectable HBV DNA by PCR, and some had elevated viral loads of up to 106 virions/ml. Acute or chronic hepatitis can be caused by HBV infection. In both stages, the hepatitis B antigen (HBsAg) is usually detected in serum first before the diagnosis is performed [36].

The research showed that there was a significant increase in the (ALP), 5' nucleotidase (5-NT), gamma glutamyl transferase (GGT), prothrombin time, (PT), alanine aminotransferase, (ALT) aspartate aminotransferase (AST), and Bilirubin in patients with viral hepatitis in comparison to the control group. Additionally, there was a decrease in the activity of serum, albumin and TSP concentration. From the results, it can be concluded that a peculiar way to diagnose viral hepatitis and to distinguish it from other types may be GGT, ALP, AST, ALT, 5-NT, and PON.

The infection results in the production of a substantial amount of HBsAg that is easily detected in some portions of HBsAg antigens representing the virions that are intact in serum. This is because a significant proportion of HBsAg consists of the antigen particles that are usually produced in excess of the intact particles [33]. The detection of HBsAg is therefore challenged by the absence of a simple tissue for culturing the virus.

Originally, HBV was detected through the recognition of the serum surface antigen (HBsAg) in individuals having HBV [34]. However, viral nucleic acids and other forms of antigenic constituents have been identified and categorized [35]. The uses of both immunometric and molecular methods have facilitated the diagnosis of HBV [36]. It is worth noting that during the selection of the right assay, there should be a close diagnostic relationship to the biological properties of the particular marker in question. Additionally, some body enzymes particularly those found in the liver and other fluids such as bilirubin make it easy for the identification of HBV based on various factors.

The superiority of PCR over other techniques such as hybridization assays has been demonstrated before. Moreover, PCR is said to detect HBV DNA in acute infections and in HBsAg-negative patients. The specificity of PCR however, may be hampered on by contamination with HBV DNA, which may arise during sample preparation, thereby resulting in false positive. Thus, extra precautions are required during sample collection and processing. In this study, all the 40 patients had detectable HBV, while none of the healthy individuals was found with HBV DNA. These findings are consistent with those of previous, which showed that HBV DNA was not detectable in normal cases. It is worth noting that HBV DNA is also not usually detectable in serum of patients who are recovering from acute HBV infection.

results of the molecular analysis were compared and correlated with the biochemical parameters including serum alkaline phosphatase, aminotransferase, albumin and bilirubin and also demographic data to evaluate the role of viral load in HBV infection. The HBV virus which belongs to the family of Hepadnaviral contains minute amounts of double-stranded DNA. Hepatitis virus is thought to have a negative effect on the liver

cells either directly or indirectly, but this effect remain unclear. In addition, the viral DNA can have a direct effect on the liver by integrating into the liver cell genome, and resulting in chromosomal instability. Chromosomal instability is associated with deletions or rearrangements of the chromosome. This integration of HBV DNA into the genome of hepatocytes can also result in dysfunction of the tumour suppressor genes and oncogenes which are involved in the survival of the cell. Furthermore, the direct effect is also linked with the 16.5kDa HBX protein that is made up of 154 amino acid. HBX protein transactivates genes that regulate cell proliferation and cell cycle as well as apoptosis and DNA repair. Management of liver cirrhosis basically involves the treatment of chronic viral hepatitis and abstinence from etiological parameters such as alcohol.

Conclusion

This study assessed the biochemical markers of the liver with specific emphasis on the significance of HBV viral load as a diagnosis parameter. Serum total bilirubin is among the first known markers for liver functioning, but still not adequate for diagnosis despite its increased activity in some liver disorders. In this study, liver damage has been attributed to high amount of bilirubin. Damaged hepatocytes produce large amounts of bilirubin resulting in high levels of bilirubin in serum. The degree of damage is dependent on the ratio of direct and indirect bilirubin. The findings indicate that most of the patients had elevated levels of bilirubin in serum suggesting necrotic tissue necrosis [37].

Despite being insufficient for a diagnosis of liver disorder, bilirubin possess a significant diagnostic value. The present study revealed high values of enzymes ALT (74-1049U/L) and AST (30-436.6U/L) in HBV patients, while normal values were detected in the normal control group. Compared with the previous findings, the results of this study are higher significantly [38]. Elevated enzyme levels occur due to increased enzyme activity which occurs when the hepatocytes are damaged. An elevated level of serum ALT is the best-known indicator of liver destruction. It has been shown that necrosis of the liver cells does not necessarily result in high levels of AST, and this explains the weak correlation that was observed between AST and HBV DNA. Previous findings have also shown that normal ALT levels and detectable HBV DNA in patients may be associated with liver damage. This was evidently shown in the present study. This study found a strong correlation between albumin, bilirubin, ALT and AST. This is in line with the findings of previous studies which showed a correlation between high levels of these parameters with liver damage, while their levels correlated well with the viral load of HCV RNA [38]. Interestingly, in the current study a strong correlation was found between ALT, AST, bilirubin and other demographic parameters with HBV DNA. High levels of serum albumin bilirubin ALT and AST has also been found in elderly individuals who are infected with the hepatitis B virus. This was evidently shown by the strong correlation that was observed between age and the biochemical parameters, as well as the HBV DNA. This reveals the role of immunity in the pathogenesis of the chronic liver disease. Molecular techniques detected very low quantities per specimen, but very low titers of serum HBV antigen in the circulation could still lead to a negative result for HBV DNA. This is because not all patients with positive HBsAg possess detectable HBV DNA. In normal cases (patients with HBV antigen but no HBV infection), the DNA is usually undetectable in serum indicating that there is no liver disease present. This study indicates that all the infected patients had detectable HBV DNA by PCR, while some had elevated viral loads. Polymerase chain reaction was able to detect extremely low amounts of HBV DNA in the circulation; all the healthy individuals had no detectable HBV DNA in their serum. However, prevailing theory behind the presence of HBs Ag with no detectable DNA is still unclear since the virions could be present in levels that are below the detection limit of PCR. Finally, more findings are required to determine whether the patients in a particular stage is in the transition to another phase or not. This is needed specifically to assess the severity of liver disease; the distinct phenotypes of HBV infection should be determined as there is a great need for antiviral therapy.

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RESEARCH PAPER

Enhance dissolution rate and solubility of solid drugs through pharmaceutical deep eutectic solvents

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A B S T R A C T:

Pharmaceutical active ingredients are usually sold in the markets in the form of solid drugs. These drugs are often associated with some problems like polymorphism, bioavailability and low solubility. In this study, deep eutectic solvents (DESs) are used to change solid drugs into liquids, which may provide a potential solution to these problems. This research describes preparation of pharmaceutical deep eutectic solvents (PDESs) from hydrogen bond donors (HBDs) like urea and glycerol and hydrogen bond acceptors (HBAs) like adiphenine HCl and ranitidine HCl, which are one of the key parts of medicine improvement. Differential scanning calorimetry (DSC) was used to measure glass transition temperatures for the prepared PDESs and fourier transform infrared spectroscopy (FT-IR) was utilized to clarify hydrogen bond formation between HBDs and HBAs. Also, dissolution test apparatus and UV-Vis spectroscopy were used to measure dissolution rate and concentration of the prepared PDESs in this work. The higher dissolution rate was achieved for the tested APIs when in the form of PDESs. This was mainly obvious for ranitidine HCl: glycerol, which was 2.6 times faster than ranitidine HCl.

KEY WORDS: Dissolution rate, Deep eutectic solvents, Adiphenine HCl and Ranitidine HCl.
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1. INTRODUCTION:

Growing attention related to the formation of novel pharmaceutical formulations has been increased significantly in health care in the world. Currently, scientists are strongly looking for new, more active and patient-compliant medicine delivery systems. Using tablets for treatment has been limited due to poor solubility of drugs and thereby bioavailability, systemic toxicity and slight pharmacokinetics (Aroso et al., 2016) . Mixtures with an active pharmaceutical ingredient (API) were used for the first time by Stott and co-workers in 1998, in their study, the authors reported mixtures of different terpenes with an API to increase skin permeation. It is described that the eutectic can enhance the solubility, absorption and permeation of the API (Stott et al., 1998).

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The subject of pharmaceutical ionic liquids has been studied (Malhotra, 2010 and Marrucho et al., 2014). In a similar way, Morrison and co-workers used malonic acid-choline chloride and urea-choline chloride as DESs to solubilize griseofulvin, benzoic acid, danazol and itraconazole. They reported that these molecules have better solubility in DESs rather than in water, in some cases, 5–22,000-fold while compared with their solubility in water (Morrison et al., 2009).

In the current study, the principle of deep eutectic solvents (DESs) is used to make liquid drugs. DESs are mixtures widely produced by combining metal halides like $SnCl_2$, $InCl_3$, $ZnCl_2$ and CuCl or HBDs such as alcohols, carboxylic acids and amides with different quaternary ammonium salts (QASs) such as choline chloride, phenformin HCl, lidocaine HCl, and imipramine HCl. HBDs interact with salts via hydrogen bonding to form DESs (Abbott et al., 2017a and Qu et al., 2021).

Currently, DESs have significantly applied in different industrial applications such as extraction of biomolecules in natural products (Abbott et al.,

2017b), extraction of nonhydrocarbon species from petroleum products and metal extraction (Smith et al., 2014). Moreover, DESs are commonly used in polymer synthesis, metal recycling, metals electrodeposition (Alesary et al., 2020), desulfurization (Qader et al., 2021) and electro polishing (Ismail et al., 2019).The concept of the current investigation was to enhance dissolution rates of drugs.

With respect to the developed Noyes-Whitney equation offers some suggestions to improve the dissolution rate of very poorly soluble drugs in order to increase their oral availability (Liu et al., 2006).The following efforts have been used to improve the dissolution rate of drugs: (a) using water-soluble transporter to form addition complexes; (b) increase the surface area by minimizing the particle size; (c) using drug derivatization and pro-drugs; (d) solubilisation in surfactant process and (e) formation of solid dispersions of drugs by minimizing their crystallinity (Liu et al., 2006). Though, in terms of practice these techniques are not easy (Kapsi and Ayres, 2001). While the dissolution rate is widely increased by decreasing particle size of compounds, there is a limitation in practice to minimize the size of particles. This can be completed by such usually used approaches as controlled grinding and crystallization. Dosage form of drugs in the form of soft powders is also problematic due to poor wettability and handling difficulties (Liu et al., 2006).

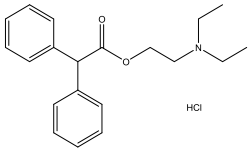
Salt formation as neutral compounds is not possible and the preparation of a proper salt form of compounds, which is weakly acidic or basic may often not be practical (Liu et al., 2006). Solid eutectic mixtures are commonly synthesized by quick cooling of a co-melt of two components to achieve a physical mixture of very soft crystals of the two compounds.

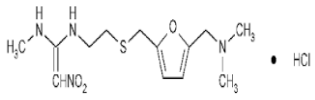
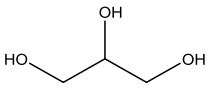
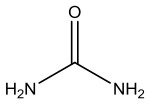
The aim of this work was to expand improving dissolution rate and solubility of two different active pharmaceutical ingredients (APIs), namely ranitidine HCl and adiphenine HCl via PDESs. These drugs were known to display polymorphism in their crystalline state. Adiphenine HCl, is a spasmolytic agent and chemically known as 2-diethylaminoethyl 2,2-diphenylacetate hydrochloride. It is used to reduce convulsion of the gastrointestinal tract, biliary tract, ureter and uterus (Dinç et al., 2014). Ranitidine HCl, is a H2-receptor antagonist with empirical formula $C_{13}H_{22}N_4O_3S$ and its chemical name is N-[*2*-[[[*5*-[(*Dimethyl amino*)*methyl]-2-furanyl*] *methyl*] *thio*] *ethyl*]-*N'-methyl-2-nitro-1,1-ethanediamine* hydrochloride. This drug aids relieve the symptoms of stomach-ache and helps the curing of ulcers (Narayana et al., 2010). Urea is used in this work, as it has many brands in the forms of urea topical, which is utilized as an emollient and useful to soften rough and dry skin as a result of eczema, keratosis, psoriasis, etc. (Celleno, 2018). Glycerol is commonly applied in the pharmaceutical industry because it can be combined into a number of pharmaceutical formulations (Abbott et al., 2017a).

2. Materials and methods

All ingredients and chemicals employed in this work were used as received and their sources and purities are listed in **Table 1.** In small test tubes quaternary ammonium salts such as ranitidine HCl and adiphenine HCl were mixed with glycerol and urea separately (molar ratios 2HBD:1QAS) to form eutectic mixtures in a similar method to that described previously (Abbott et al., 2017a).

Table 1: Structure, sources and purity of chemicals used in this study.

Chemicals	Structure	Source	Purity %
Adiphenine hydrochloride		Sigma-Aldrich	≥99
Hydrochloric acid	HCl	Chem-Lab	37

Ranitidine hydrochloride		Sigma-Aldrich	CAS 66357-59-3
Glycerol		Scharlau	99
Urea		Fluka	≥99.5

All mixtures were put in an oven at 50 ^{o}C for 24 hours after that, the magnetic stirrer hotplate was used to heat these mixtures at approximately 80 ^{o}C and 600 rpm for at least 2 hours until clear homogenous liquids were formed for each.

The produced PDESs are liquids at 25 ^{o}C and their glass transition temperatures were measured by a Mettler Toledo Differential Scanning Calorimetry (DSC) from the chemistry department- University of Leicester- United Kingdom.

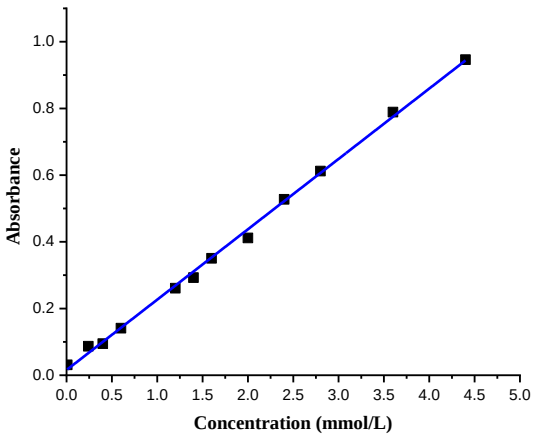
A PerkinElmer, Shelton, CT 06484 USA Lambda 25 UV/VIS Spectrophotometer was used for all UV-Vis absorption amounts. A number of standard solutions were prepared from pure drugs (adiphenine HCl and ranitidine HCl) in 0.1 M HCl separately. The absorbance measured for each solution by UV-Vis spectroscopy. Calibration curves were made in 0.1 M HCl in different concentrations and the results are shown in **Fig. 1** for ranitidine HCl and adiphenine HCl. In this case, the points on the graph made a straight line by using OriginPro 2018 64- bit. The intercept and slope of the produced line form a correlation between concentration and absorbance.

Absorbance = slope * concentration + intercept (1.1)

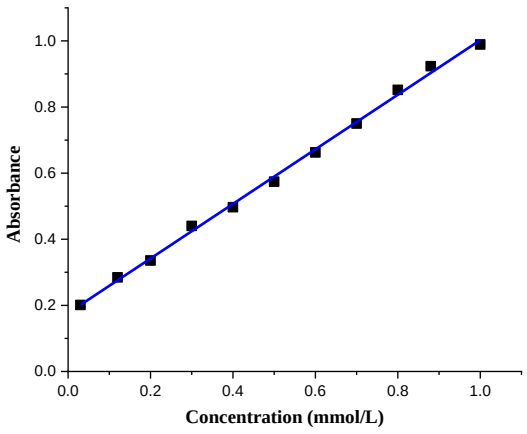
The concentration of adiphenine HCl and ranitidine HCl in their PDESs is measured from the calibration curve. This can be conducted by determine the absorbance of the active ingredients in their PDESs. This absorbance is used with the intercept and slope from the calibration curve to determine the concentration of unknowns' solution.

Concentration = (absorbance – intercept) / slope (1.2)

Two calibration curves for adiphenine HCl and ranitidine HCl were made separately in the range of 0.008 - 4.4 and 0.201 - 0.9891 mmol L^{-1} respectively. The sample concentrations were diluted to be ensure that they were constantly in these ranges.



(a)



(b)

Figure 1*: Calibration curves using standard known a) adiphenine HCl concentrations in 0.1M HCl which are plotted against absorbance peaks in UV-Vis spectroscopy, b) ranitidine hydrochloride concentrations in 0.1M HCl which are plotted against absorbance peaks.*

PHARMA TEST PT-DT7, dissolution apparatus type Ⅱ with paddle stirrer was used to study the dissolution rate of pure PAIs like adiphenine HCl and ranitidine HCl and the PDESs prepared from these PAIs in 0.1 mol/L HCl (pH=1.0) at 37 ± 0.5 °C (to mimic the behaviour in the stomach).

Furthermore, Fourier-transform infrared spectroscopy (Perkin Elmer Spectrum One FT-IR with ATR)) was applied for the produced PDESs to compare the constituents with their products via hydrogen bonding. Hydrogen bond formations between HBDSs and HBAs were showed using this machine. Thus, hydrogen bond formations can be predictable and investigated via FT-IR. FT-IR is a powerful method to obtain data of hydrogen bonding.

3. Results and discussion

3.1 UV-Vis spectroscopy study

It can be noticed that good linear correlation was carried out for this analytical technique, the R^2 value was more than 0.99 for UV-Vis spectroscopy in all cases. The error bars for most of the results are within the size of the plot symbols displaying that replicate data are accurate. The reproducibility of replicate determinations can again be noticed from the small error bars on the curve. Adiphenine HCl and ranitidine HCl absorbed at around 258 and 314 nm respectively on UV-Vis spectroscopy in 0.1M HCl.

The prepared PDESs are liquid at room temperature as shown in **Fig. 2**. DSC was used to determine glass transition temperatures of adiphenine HCl and ranitidine HCl at their PDESs: (adiphenine HCl + urea), (adiphenine HCl + glycerol), (ranitidine HCl + urea) and (ranitidine HCl + glycerol) were -45, -91, -51 and -87 °C separately. The results show that the solubility and dissolution rate, release profile of these liquids enhanced and may increase the bioavailability.

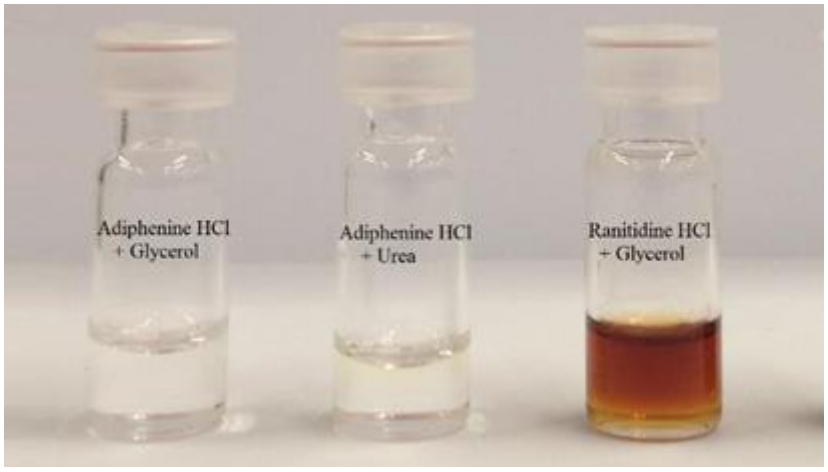


Figure 2*: Pharmaceutically active ingredients as quaternary ammonium salts formulated into DESs in a 1 QAS: 2 HBD molar ratio.*

Fig. 3 shows that pure active ingredients (adiphenine HCl and ranitidine HCl) and the prepared PDESs from these ingredients were absorbed at the same wave length range without shifting. This indicates that pharmaceutical active ingredients kept their pharmacological effects in their new formulations.

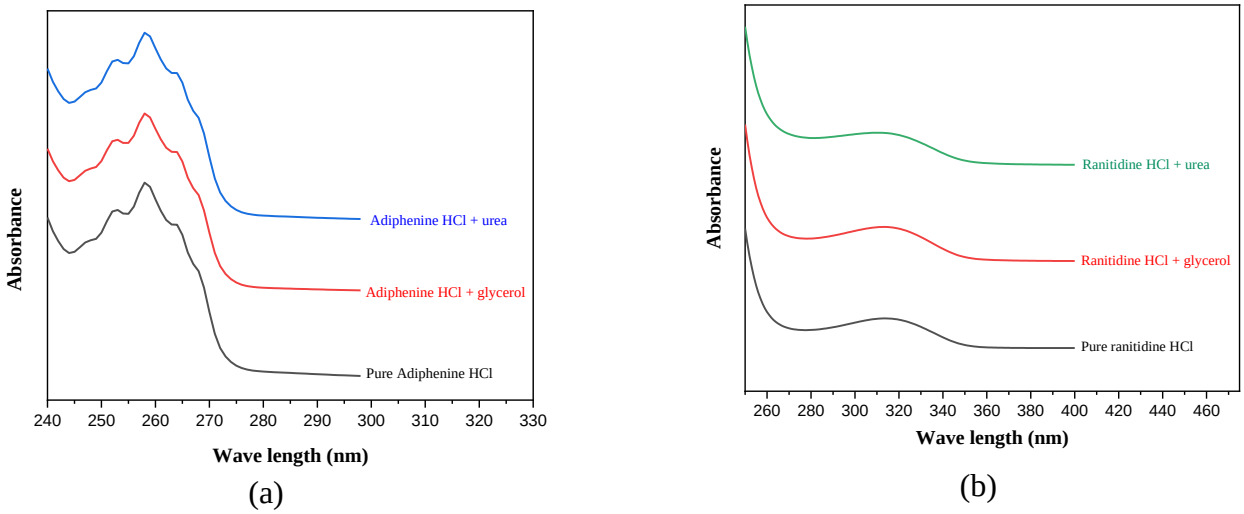


***Figure 3**: Absorption spectra of (a) (Adiphenine HCl, Adiphenine HCl + glycerol and Adiphenine HCl + urea) in 0.1mol /L HCl and (b) (Ranitidine HCl, Ranitidine HCl + glycerol and Ranitidine HCl + urea) in 0.1mol /L HCl.*

3.2 Dissolution rate study

In this study, adiphenine HCl and ranitidine HCl were used as hydrogen bond acceptors with urea and glycerol as hydrogen bond donors to form PDESs. The increase in dissolution rate was achieved for the produced PDESs. For example, ranitidine HCl is a polymorphic drug in the form of solid state which its dissolution rate unstable. Thus, PDES from ranitidine HCl: urea and ranitidine HCl: glycerol achieved higher dissolution rates than pure ranitidine HCl as shown in **Fig. 4**.

Aliquots of the HCl solution were used as a function of time and the PDES concentration in solution was measured using UV-Vis spectroscopy using the calibration curves demonstrated above.

PDESs such as (adiphenine HCl + urea), (adiphenine HCl + glycerol), (ranitidine HCl + urea) and (ranitidine HCl + glycerol) performed to be faster dissolution rates than pure adiphenine HCl and ranitidine HCl respectively**.** **Fig. 4** shows the summaries of their release rate are demonstrated as the percentage of the pure drug as a function of time. It should be mentioned that these experiments were conducted with very slow magnetic stirring which is why the PDESs are not immediately miscible.

It was shown that the dissolution rates of adiphenine HCl and ranitidine HCl at their eutectic preparations: (adiphenine HCl + urea), (adiphenine HCl + glycerol), (ranitidine HCl + urea) and (ranitidine HCl + glycerol) were 2.0, 2.5, 2.3 and 2.6 times faster than their pure drugs respectively.

Touitou *et al*. and Kasting *et al* (Stott et al., 1998) displayed that the solubility of drugs depends on their melting points. They showed that the solubility and transdermal permeation increases by reducing the melting point of a drug molecule. Thus, eutectic formation can be applied as a process to reduce the melting point of PAIs with keeping their pharmaceutical activity (Stott et al., 1998). This section can be concluded that the decrease of melting point causes to increase dissolution rate, solubility and hence increase the bioavailability.

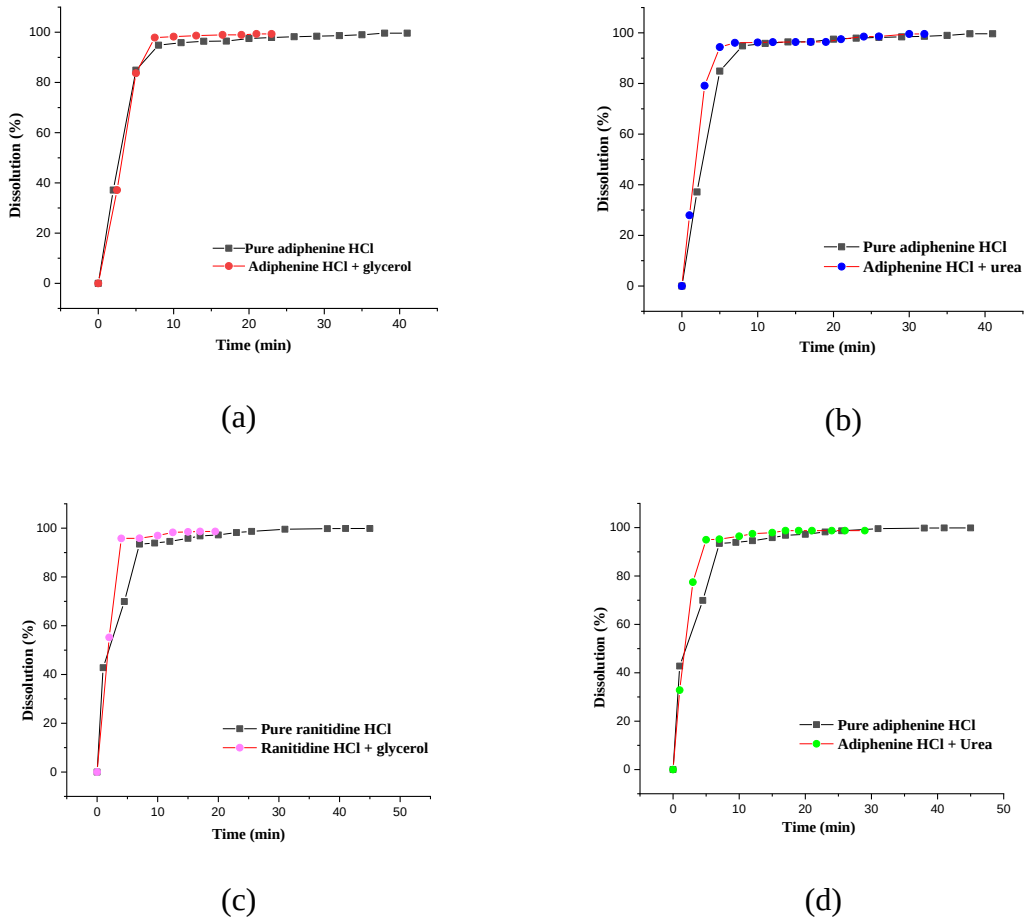


Figure 4*: a) Adiphenine HCl + glycerol (0.1223 g) in 0.1mol /L HCl at 37 ± 0.5°C, b) Adiphenine HCl + urea (0.1063 g) in 0.1mol /L HCl at 37 ± 0.5°C , C) (ranitidine HCl + glycerol (0.0677g) in 0.1mol/L HCl at 37 ± 0.5°C and d) (ranitidine HCl + urea (0.0596g) in 0.1mol/L HCl at 37 ± 0.5°C.*

3.3 Fourier-transform infrared spectroscopy (FT-IR)

In the present work Fourier-transform infrared was used as a potent method to obtain data of hydrogen bonding. Hydrogen bonding has a significant effect on the OH stretching vibration in HBDs (Hou et al., 2015). **Fig. 5** shows FT-IR spectra of glycerol and PDES formed by glycerol and ranitidine HCl. The OH stretching vibration of pure glycerol was observed at 3299 cm^{-1} which shifted to 3257 cm^{-1} in the PDES produced by glycerol and ranitidine HCl. The FT-IR spectra of the HBDs in the PDESs indicate that the shift in vibrational state happened because a portion of the cloud of electrons of the oxygen atom transfers to the hydrogen bonding, decreasing the force constant (Hou et al., 2015 and Aissaoui et al., 2015).

Hence, the shift of the OH stretching vibration proposes the present of hydrogen bond between glycerol as the HBD and ranitidine HCl as the HBA, when the PDESs were formed. In addition, the FT-IR spectra showed broader peaks for the produced PDESs because of the existence of hydrogen bonding.

Furthermore, FT-IR was also used to further study the interaction between ranitidine HCl and urea, and identify the structure of ranitidine HCl – urea mixtures. The FT-IR spectra of solid urea and the eutectic mixture ranitidine HCl – urea were conducted at room temperature. Compared with individual components, it demonstrated that the absorption band at 3429 cm^{-1} in urea was shifted to 3334 cm^{-1} in ranitidine HCl – urea as shown in **Fig. 6**.

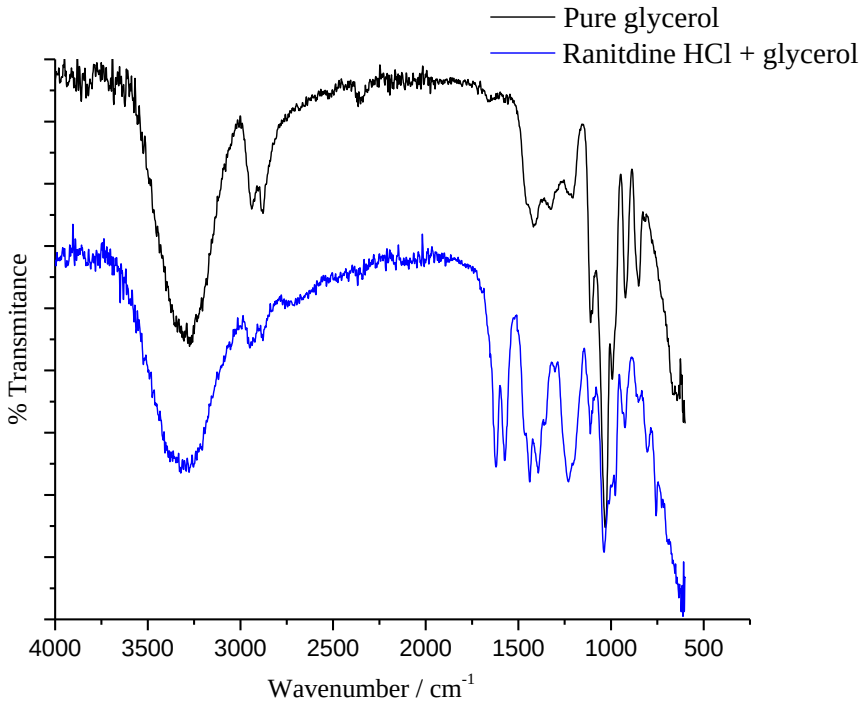


***Figure 5**: FT-IR spectrum of pure glycerol and (ranitidine hydrochloride + glycerol) with molar ratio (1:2).*

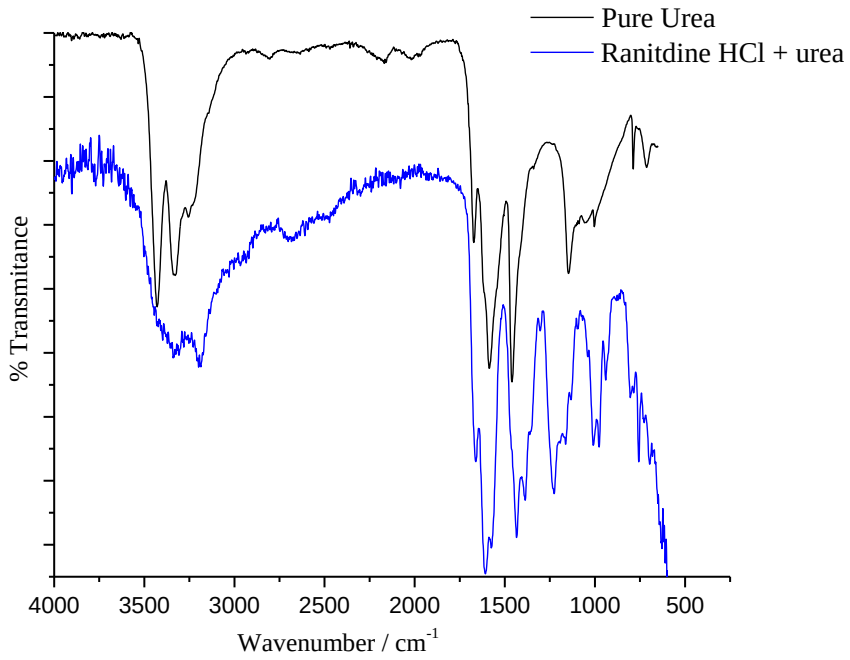


***Figure 6**: FT-IR spectrum of pure urea and (ranitidine hydrochloride + urea) with molar ratio (1:2).*

Hence, the shift of the NH stretching vibration proposes the present of hydrogen bond between urea as the HBD and ranitidine HCl as the HBA, when the PDESs were formed. Moreover, the FT-IR spectra showed broader peaks for the produced PDESs because of the presence of hydrogen bonding.

This in agreement with the study described by Hua Wang *et al* (Zeng et al., 2016) for a formation of DES between urea as the HBD and betaine. In addition, the present study is in agreement with the observation recorded by Aissaoui *et al* (Aissaoui et al., 2015) for DESs prepared by mixing triethylenglycol, diethylenglycol, ethylenglycol and glycerol as HBDs with methyltriphenylphosphonium bromide as a salt. **Table 2** shows the shift of the OH and NH stretching vibrations of (glycerol and urea) and the produced PDESs from these HBDs.

Table 2: The shift of OH and NH stretching vibration of the HBDs.

HBDs	QASs	HBDs: QASs	Wavenumber (cm^{-1}) of pure HBDs	Wavenumber (cm^{-1}) of HBDs + QASs
Glycerol	Ranitidine HCl	2:1	3299	3257
Glycerol	Adiphenine HCl	2:1	3299	3271
Urea	Ranitidine HCl	2:1	3429	3334
Urea	Adiphenine HCl	2:1	3429	3297

4. Conclusion

This research has shown that pharmaceutical active ingredients can be formulated into liquids as PDESs. These active ingredients were known to show polymorphism in the crystalline form. The main point to note is that the PDESs were used as a design strategy to change solid drugs into liquids, which should overcome these issues associated with solid drugs, such as polymorphism, bioavailability and solubility. The compounds selected were adiphenine HCl and ranitidine HCl and the hydrogen bond donors were glycerol and urea. The prepared PDESs have glass transition temperatures which were measured by DSC. In addition, FT-IR was used for the formed PDESs to compare the components with their products via hydrogen bonding. One of the most interesting aspects in this research is the study of the dissolution rate of PDESs. The purpose of this was to improve the dissolution rates and solubilities of adiphenine HCl and ranitidine HCl via PDESs. PDESs based on urea or glycerol mixed with these APIs, were prepared and characterized for dissolution rates and solubilities. The results obtained show higher dissolution rates of the APIs when in the form of PDESs. The increase in dissolution rate was mainly obvious for ranitidine HCl: glycerol. Thus, PDES can be encouraging for increasing dissolution rate and solubility of poorly soluble drugs.

This research has recommended not only that solid drugs can be changed into liquids and increase their dissolution rates, but the capability to formulate the PDES into a gelatin tablet will be studied as well. In addition to that the delivery of drugs from patches by using PDESs can be studied.

Acknowledgements

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RESEARCH PAPER

Molecular Cytogenetic Study in Patients with Acute Lymphoblastic Leukemia (ALL) in Erbil Province

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A B S T R A C T:

Background: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and is also important in older adults. Chromosome number or structure abnormalities are seen in approximately 90% of children and 70% of adult patients with ALL. The aim of this study was to determine the prevalence of these chromosomal abnormalities in ALL patients using Fluorescence *in situ* Hybridization (FISH) technique and to define the frequency of chromosomal abnormalities of ALL patients in adults and children in Erbil Province.

Methods: In this cross-sectional study, we evaluated karyotype results in blood samples, that collected from 55 patients with ALL (in both sexes) in Nanakali Hospital in Erbil Province. Thirty healthy individuals were selected as the control group. Patients ages ranged between 1 to 63 years old. The samples were centrifuged to extract nucleated cells. The cells were then subjected to hypotonic shock, fixed with methanol and acetic acid. A cell suspension was then prepared for FISH technique. After examining the samples with fluorescent microscope, the obtained data along with demographic and baseline characteristics of patients were entered in SPSS software, then statistically analyzed.

Results: The prevalence of chromosomal abnormalities among ALL group was 52.7% (n = 29). Of these, 31¬% (9 people) had abnormalities in chromosome number and 69¬% (20 people) had abnormalities in chromosome structure. The most common chromosomal abnormality was translocation t (9; 22), which accounted for 31¬% of all abnormalities and its prevalence among ALL patients was 16.4¬%. Clonal trisomy and t (12; 21) also accounted for 13.8% and 10.3% of abnormalities, respectively. Clonal trisomy was the most common abnormality in chromosome number, accounting for 44.4% (n = 4) of abnormalities. Only one patient with single chromosome X (X0) pattern was observed in patients. There was no significant (P> 0.05) relationship between the incidence of chromosomal abnormalities with gender, family history, history of surgery and bacterial infection, occupation, place of residence, smoking and blood type as stated from the questionnaire form.

Conclusion

In the current study, concluded that at least one chromosomal abnormality was found in more than half of all patients with ALL. Structural abnormalities were more common than chromosome number abnormalities. Awareness of the magnitude of the problem demands implementation of preventive, diagnostic and therapeutic strategies for leukemia's in the Kurdistan region as well as planning epidemiologic studies and research programs.

KEY WORDS: Acute Lymphoblastic Leukemia,Cytogenetic Analysis,Molecular Cytogenetic Analysis, Chromosomal Abnormalities, Fluorescence in situ Hybridization(FISH), Erbil, Iraq.
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1. INTRODUCTION:

Acute lymphoblastic leukemia is characterized by excessive production of immature lymphocytes (lymphoblast) in the bone marrow preventing normal hematopoiesis.

If untreated ALL can cause death due to crowding out normal cells in the bone marrow and by metastasizing to other essential organs through the peripheral blood, the lymph nodes, spleen, liver, central nervous system (CNS), and skin are the most commonly diagnosed organs. Symptoms are caused by varying degrees of anemia, neutropenia, and thrombocytopenia, or the penetration of ALL cells into tissues. Acute lymphoblastic leukemia is the most common malignant disease in children

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with a peak incidence between the ages of 2 -5 (Terwilliger & Abdul-Hay, 2017). It also is associated with about 20% of acute adult leukemia patients. Although the peak incidence of ALL is early in life, 45% of patient are nonetheless diagnosed in adulthood (over 20 years). The global prevalence of ALL varies between 1 and 5 per 100,000 individuals and is slightly higher in men than women (Katz et al., 2015). The prevalence of this disease is higher in Latin countries and Spain and generally industrialized countries and urban areas. It is more common in Caucasians than African Americans (Kakaje et al., 2020).

For the correct diagnosis and classification of ALL, the morphologic recognition and phenotypic characteristics of lymphoblast's in the blood and bone marrow are also essential. These cases require accurate evaluation of peripheral blood and bone marrow samples with appropriate preparation and phenotypic analysis of blasts through flow cytometry and immunohistochemistry methods, using an appropriate plate and cytoplasmic markers (Gurbuxani et al., 2020). The results of peripheral blood and bone marrow lymphoblast surveys can be varied. A definitive diagnosis of ALL is based on bone marrow biopsy and identification of malignant clones in flow cytometry with a distinction between B and T cell cancers. ALL B cells account are identified by the expression of CD19, CD22, and CD79a (Raponi et al., 2011).

This invasive form shows a survival rate of 80-90% with chemo immunotherapy. There are some specific recurrent chromosomal abnormalities in ALL patients that are important in determining the prognosis of the disease for treatment planning. Abnormalities in chromosome number or structure are encountered in approximately 90% of children and 70% of adult ALL patients (Gurbuxani et al., 2020). The most common chromosomal shifts in ALL are t (9; 22) (190p) in adults and t (12; 21) in children, respectively. The most common numerical disorder in ALL is hyperdiploidy with chromosome numbers from 51 to 63, which occurs with trisomy of chromosomes X, 4, 6, 10, 14, 17, and 18 and chromosome 21 of four copies. These cytogenetic abnormalities are caused by somatic cell mutations (instead of germ cell mutations), which are often caused by chromosomal DNA translocation, resulting in new (abnormal) protein products from the combined genes.

Cytogenetic analysis is the standard tool for initial evaluation, diagnosis, management of hematological malignancy of a patient that suspected to cancer (Goh et al., 2006) and used as a prognostic indicator for monitoring therapy (Parikh and Tefferi, 2012). Also, it provides evidence of the progression of disease at an earlier phase than hematological marker by detecting various chromosomal aberrations.

The advent of the FISH technique in the 1980s revolutionized cytogenetic analysis. The FISH method was introduced as a technique for identifying trisomy's and displacements in metaphase and interphase nuclei using DNA libraries. This technique is a very good tool for studying the structure and function of chromosomes, polyploidy, aneuploidy, foreign gene penetration, and genome evolution, and for physical mapping of genes. In many applications, in situ hybridization (ISH) requires effective methods for chromosome preparation where chromosomes have to be well-preserved and properly distributed (Liehr, 2017). FISH uses fluorescently labeled DNA probes to determine chromosomal positions within the nucleus. Fluorescent materials generate color signals and are detected using a fluorescent microscope.

Recent changes in the WHO classification identify specific types of B-ALL with recurrent cytogenetic abnormalities (Swerdlow et al., 2017). Translocation (13p; 23q) t (1; 19) is one of the most common translocations that take place in both adult and juvenile populations with an overall frequency of 6%. This shift is observed in the B-ALL field Hyperploid, which is associated with a poor prognosis. The shift can also occur in balanced forms - (13p; 23q) t (1; 19) or unbalanced - (13p; 23q) t (1; 19) der (19) and can lead to integration (Shago, 2017). The current study was aimed to evaluate and the cytogenetic and molecular evaluation of patients with acute lymphocytic leukemia (ALL) using *in situ* fluorescence hybridization (FISH), prevalence of chromosomal translocations in patients with acute lymphocytic leukemia and to determine the frequency of different translocations and comparing their frequency and relationship between chromosomal displacement and risk factors in patients with acute lymphocytic leukemia (Alghasi A, et al., 2019).

2.MATERIALS AND METHODS

2.1Participants

This current retrospective cohort study was conducted on 55 patients & 30 healthy individuals & it current out from January 2020 to August 2020, in Nanakali hospital for blood diseases and oncology the current study cases were reviewed with a final diagnosis of ALL. Definite diagnosis in all cases was established based on morphology, cytochemistry, histochemistry, and flow cytometric analysis. All the cases were referred from affiliated hospitals in Nanakali Hospital for Blood Diseases and Cancer, Erbil, Iraq. Fesh peripheral blood samples were collected.

Briefly, peripheral blood samples were cultured in RPMI 1640 basal medium, containing 10% fetal calf serum (Gibco Invitrogen-USA), for 72 hours at 37°C, at 45□ angle for an increased growth surface with frequent smooth shaking every 24 hours. Then treated with 100 micrograms/ ml of colcemid (Gibco-Invitrogen-USA) to stop the cells in the metaphase of mitosis. After harvesting with hypotonic solution (0.075 M KCL) and fixation with acetic acid /methanol (1/3), the chromosomes were spread and stained using the standard G-banding technique was performed by treating the prepared slides with trypsin working solution and Giemsa stain solution (Moorhead *et al*., 1960). For each case, a minimum of 20 metaphases was analyzed by using the CytoVision chromosomal karyotyping automatic system (Genetix CompanyUSA). Karyotype was written according to the International System Chromosome Nomenclature (ISCN). A successful cytogenetic analysis required the detection of at least 2 or more cells with the same structural change or chromosomal gain, 3 or more cells with the same chromosomal loss, in at least 20 metaphases (Qureshi, 2008). The patients' karyotypes were thereafter subdivided into groups based on the WHO classification.

For the molecular cytogenetic study, a total of (3-5) ml of venous blood was collected from 15 patients, ages ranged between 4 to 52 years with ALL and 5 healthy individuals were selected as the control group. The FISH technique was performed according to (Llobet-Brossa et al., 1998) where the FISH probes were reversibly connected to a glass device and then contacted with the hybridization buffer hardened from the saline-sodium citrate (SSC) solution. After dissolving the probes in the solution, denaturation of the probes and target DNAs was performed by heating the solution at 78 ° C for 5 minutes. Hybridization conditions were provided by placing the samples at 42 ° C for 14-16 hours. After hybridization, the samples were combined with Diamidino-2-phenylindole (DAPI) and allowed to produce color in the dark. Finally, the samples were placed on a slide and examined under a fluorescent microscope.

A multi-probe panel was designed for ALL to identify FISH probes including BCR / ABL translocation, mixed cell line leukemia rearrangement (MLL), TEL-AML1 gene fusion, and trisomy 4.10. The probes were located in regions 11.2q22 34q9, 23q11, 13p12 22q21 and 11.1q -11.1p10 / 11.1q -11.1p4, which represent chromosomal abnormalities (11q; 34q) t (9; 22), 23q11, (22q; 13q) t (12; 21), respectively, and trisomy were 4.10.

Demographic characteristics are examined in two groups of control and patients with ALL. Demographic and baseline information inspected includes age, gender, occupation, family history, maternal and paternal kinship, place of residence, history of bacterial infection and surgery, cytogenetic assessment method, smoking, and blood type as came from the questionnaire form.

3.RESULTS

The current study conducted a cytogenetic analysis on 55 ALL patients with the age ranged between 1-63 years. The prevalence of chromosomal abnormalities in the control and patient groups were investigated according to their type and the prevalence of each type was reported separately as shown in (Table 1).

The gender distribution of subjects in the control and experimental groups of males and females are 60% and 40% respectively, as represented in (figure 1). In terms of gender, 60% of participants were male and the remainders were females, 69.4% of the participants were live in Erbil. In terms of genetic abnormality, karyotyping was used in 76.5% of cases, and FISH were used in 23.5%.

The mean age of the whole population was 15.42. The mean age of the control group was15.93, and the mean age of the experimental group was 15.14. These findings indicate that both groups were well adjusted together in terms of age

(Table 2). In both groups, the most common age groups were 1 to 10 years, 11 to 20 years and 21 to 30 years, and their percentage values were 4.11% and 47.3% respectively. No one in the present study was between 41 and 50 years old.

While according to (Figure 2), karyotyping in both groups was 16.7% and 27.3%, respectively. While the percentages of Karyotyping using the FISH were 83.3 and 72.7 in both control and patient groups respectively.

Genetic abnormalities were assessed in 5 control group members and 15 experimental group members, using the FISH method. 25 members in the control group and 40 members in the experimental group were also tested with karyotyping. Examination of the results of FISH and karyotyping revealed no chromosomal abnormalities in the control group, and therefore all cases belong to the experimental group (ALL patients).

The prevalence of chromosomal abnormalities among people with ALL was 52.7% (n = 29), which means that more than half of the patients showed at least one chromosomal abnormality. Of these, 31% (9 individuals) had abnormalities in chromosome count and 69% (20 individuals) had abnormalities in chromosome structure (Table 3). 36.43% of patients with ALL showed structural abnormalities and 16.4% showed abnormal chromosome counts (Figure 3).

The most common chromosomal abnormality was translocation t (9; 22), which accounted for 31% of all abnormalities. Its prevalence among ALL patients was 16.4.%. “Clonal trisomy” and “t (12; 21)” accounted for 13.8% and 10.3%, respectively (Table 4). Table 5 showed the prevalence of chromosomal abnormalities in patients with ALL by type of abnormality. Clonal trisomy was the most common abnormality in chromosome number, accounting for 44.4% (n = 4) of abnormalities. Only one case of a single X (XO) pattern was observed in patients. The most common chromosome structure abnormalities were “t (9; 22)” and then “t (12; 21)”, which accounted for 45% and 15% of structural abnormalities, respectively. The prevalence of structural abnormalities was recognized in patients with ALL by type of mutation. A total of 20 structural abnormalities were reported, of which 70% were displacement, 25% were omission, and 5% were doubling (Table 6).

Table 1: Demographic characteristic, basic information, abundance and frequency of the subjects

	Measures	**Abundance**	**Frequency**
Gender	Male	51	60
	Female	34	40
Cytogenetic method	FISH	20	23.5
	Karyotype	65	76.5
Group	Control	30	35.3
	ALL test	55	64.7
Job	Jobless	29	34.1
	Student	39	45.9
	Freelance	14	16.5
	Employee	3	3.5
Location	Erbil	59	69.4
	Outside of Erbil	26	30.6

family history	Yes	8	13.3
	No	52	86.7
Affected relatives	Yes	24	28.2
	No	61	71.8
Bacterial infection	Yes	15	27.3
	No	40	72.7
Smoking	Yes	13	16.2
	No	67	83.8
History of surgery	Yes	7	8.2
	No	78	91.8
Blood group	A	16	18.8
	B	27	31.8
	O	31	36.5
	AB	11	12.9

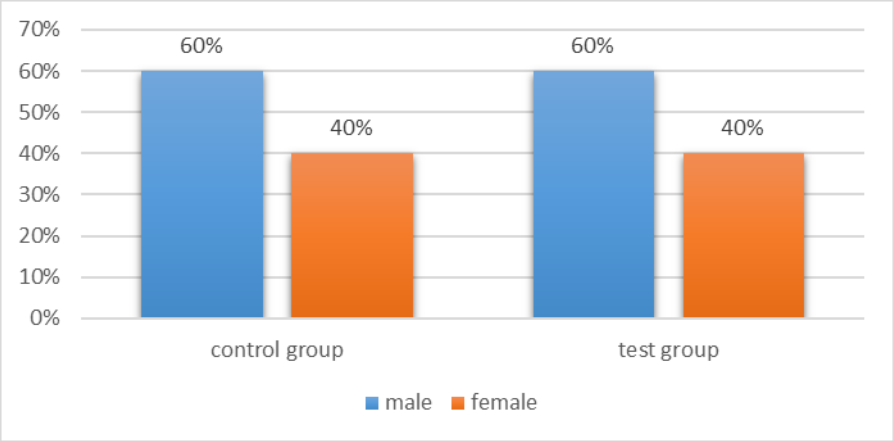

Figure 1: Gender distribution of subjects in the control and experimental groups.

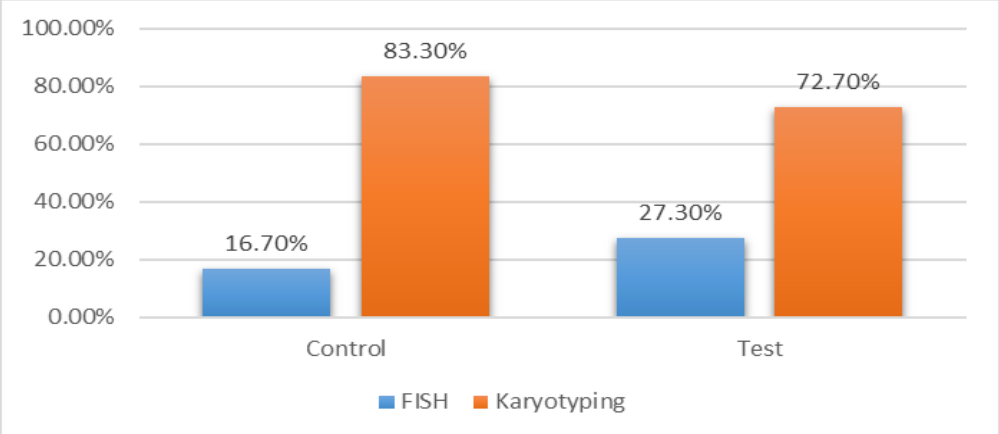

Figure 2: Type of cytogenetic method used to determine chromosomal abnormalities in control and experimental groups.

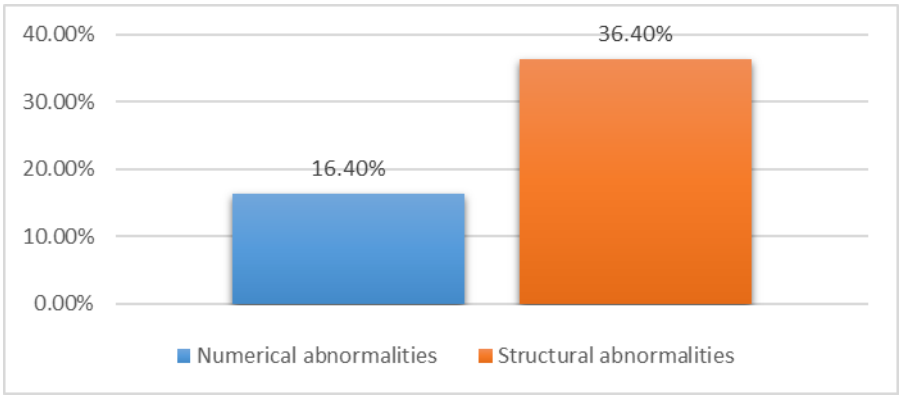

Figure 3: Prevalence of chromosomal abnormalities and type of abnormalities in ALL patients

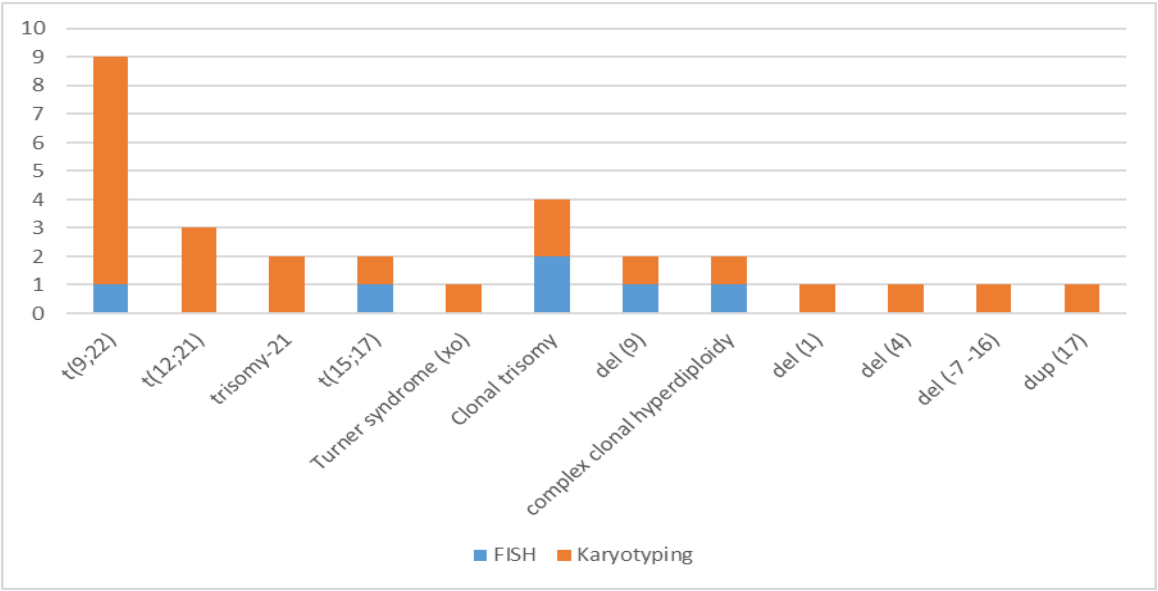

Figure 4: Frequency of chromosomal abnormalities in patients with ALL by cytogenetic studies.

Table 2: The average age of the subjects by groups

	Number(n)	**Average (Mean)**	**S. D**	**Minimum (Year)**	**Maximum (Year)**
Control	30	15.93	13.61	1	63
Test	55	15.14	13.20	1	63
Total	85	15.42	13.27	1	63

S.D = Standard Deviation

Table 3: Prevalence of chromosomal abnormalities and type of abnormalities in ALL patients with cytogenetic studies

		Abundance	Frequency	Prevalence in patients
Chromosomal abnormalities	Yes	29	52.7	52.7
	No	26	47.3	47.3
Type of chromosomal abnormality	No.	9	31	16.4
	Structure	20	69	36.4

Table 4: Prevalence of chromosomal abnormalities in patients with ALL with cytogenetic studies

Chromosomal abnormalities	Abundance	Frequency	Prevalence in patients
t(9;22)	9	31	16.4
t(12;21)	3	10.3	5.5
Trisomy 21	2	6.9	3.6
t(15;17)	2	6.9	3.6
Turner syndrome (xo)	1	3.4	1.8
Clonal trisomy	4	13.8	7.3
del(9)	2	6.9	3.6
complex hyperdiploidy cancer clonal	2	6.9	3.6
del(1)	1	3.4	1.8
del(4)	1	3.4	1.8
del(16- 7-)	1	3.4	1.8
dup(17)	1	3.4	1.8

Table 5: Prevalence of chromosomal abnormalities in patients with ALL by type of abnormality

Type of chromosomal abnormality		Abundance	Frequency
Anomalies Number of chromosomes	Trisomy 21	2	22.2
	Single X (xo)	1	11.1
	Clonal trisomy	4	44.4
	complex hyperdiploidy cancer clonal	2	22.2
	Total	9	100
Anomalies Chromosome structure	t(9;22)	9	45
	t(12;21)	3	15
	t(15;17)	2	10

	del(9)	2	10
	del(1)	1	5
	del(4)	1	5
	del(16- 7-)	1	5
	dup(17)	1	5
	Total	20	100

Table 6: Prevalence of structural abnormalities in patients with ALL by type of mutation

Type of mutation	**Abundance**	**Frequency**	**Prevalence in patients**
displacement	14	70	25.5
Delete	5	25	9.1
To be doubled	1	5	1.8
Total	20	100	36.4

4.DISCUSSION

The current study findings revealed that the prevalence of chromosomal abnormalities among people with ALL was 52.7% (n = 29), which means that more than half of the patients showed at least one chromosomal abnormality. Of these, 31% (9 individuals) had abnormalities in chromosome number and 69% (20 individuals) had abnormalities in chromosome structure, the

prevalence of this anomaly among ALL patients was reported to be 16.4% and the prevalence of chromosome structure abnormalities was 36.4%, which was consistent to the findings reported by (Moorman et al.,2010), and his prevalence of genetic abnormalities was 74%. While in another study done by Shaikh et al. (2014) investigated chromosomal abnormalities in children under 15 years of age with ALL. In this study, which examined a total of 153 children with ALL, the prevalence of chromosomal abnormalities was reported to be 48.8%. This may belong to the difference in the sample size of the study.

In the current study, the most common chromosomal abnormality was translocation t (9; 22), which accounted for 31% of all abnormalities. Clonal trisomy and t (12; 21) accounted for 13.8% and 10.3%, respectively. Clonal trisomy was the most common abnormality in chromosome number, accounting for 44.4% (n = 4) of abnormalities. Only one case of a single X (X0) pattern was observed in patients. The most common chromosome structure abnormalities were t (9; 22) and then t (12; 21), which accounted for 45% and 15% of structural abnormalities, respectively. A total of 20 structural abnormalities were reported, of which 70% were displacement, 25% were omission and 5% were doubling. 25.5% of the subjects had chromosomal abnormalities of displacement type, 9.1% of deletion type, and 1.8% of duplication, t-displacement (9; 22) was the most common structural chromosomal abnormality and accounted for 16.4% of cases.

According to a study evaluated the cytogenetics of patients with ALL, the prevalence of t-shift (9; 22) among large sample size (236 patients) was 15%. This translocation was the most common chromosomal disorder in ALL patients (Moorman et al., 2010).

Although, in another study done by Shaikh et al. (2014), 14.2% of chromosomal abnormalities were of the displacement type, 4.72% of the deletion type, and 7.87% of the duplication type performed on children under 15 years of age. The most common chromosomal abnormality was hyperploidy (13.4%) followed by displacement t (9; 22) (7.08%) was the most common structural disorder This difference in the prevalence of

structural disorders is probably due to differences in the study population.

A study was performed by Roberts et al. (2017) on 798 patients with ALL ranging in age from 21 to 86 years. This study showed that the Philadelphia chromosome abnormality t (9; 22) is about 20% among these individuals, which was in agreement with the current study results. The difference observed in the results can be due to differences in sample size and age group of the subjects.

A study by Reddy et al. (2019), evaluated genetic abnormalities in 204 patients with ALL. The most common karyotypes observed include normal karyotype in 39.7% (n = 81), hyperdiploidy in 12.7% (n = 26), t (9; 22) in 4.4% (n = 9), and t (1; 19) in 3.9%. (8 people), and normal karyotype was observed in 47.3% of patients, and t (9; 22) abnormality was reported in a larger population. This discrepancy in the results may be due to differences in the geographical area of the subjects or the sample size.

A study by Chennamaneni et al. (2018) on the cytogenetic effect on treatment outcomes and survival of children with ALL. A total of 240 patients under the age of 18. Out of 240 patients, 125 (52%) were cytogenetically evaluable. Of these, 77 patients (61.6%) had normal cytogenetics, 19 patients (15.2%) had undesirable t (9; 22), 10 patients (8%) had unfavorable cytogenetics, including t (9; 11), hypodiploidy and the karyotype was complex, 10 patients (8%) had favorable cytogenetics including t (12; 21), t (1; 19) and hyperdiploidy, 9 patients (7.2%) had different cytogenetics (Chennamaneni et al., 2018). In the above study, more than half of the subjects had a normal karyotype, while in the present study, less than half of the subjects had a normal karyotype, and the results are not consistent. The difference might be because the above study was performed on individuals under 18 years of age and the present study included adults.

5.CONCLUSION

the current, study concluded that at least one chromosomal abnormality was found in more than half of the patients with ALL. There was no relationship between demographic and baseline information such as gender, age, blood type, family history, etc. with the occurrence of chromosomal abnormalities. Structural abnormalities were more common than chromosome number. Awareness of the magnitude of the problem demands implementation of preventive, diagnostic and therapeutic strategies for leukemia's in the Kurdistan region as well as planning epidemiologic studies and research programs. Extensive studies with larger sample sizes are required in this area.

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RESEARCH PAPER

Arterial blood gases and some blood parameters in Tetralogy of Fallot patients

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A B S T R A C T:

The present study was done to assess the effect of the tetralogy of Fallot (TOF) on some arterial blood measurements including pH, the partial pressure of carbon dioxide (PaCO2), the partial pressure of oxygen (PaO2), Base excess (BE), Red blood cells (RBC), Packed cell volume (PCV), and Hemoglobin (Hb) concentration in the Erbil City of Iraq. For this purpose 80 (40 males and 40 females) patients with (TOF) and aged between 3-33 years were used in this study. This retrospective cohort study was conducted from March /2018 to October 2020 and carried out in the Surgeries Hospital Specialist - Cardiac Center in Erbil city. The studied parameters were estimated before and two to three weeks after postoperative correction of TOF. A dependent t-test was used for data analysis. The results showed that males and females with (TOF) after surgical correction have significantly $p \leq 0.001$ lower PaCO2, RBC, PCV, and Hb concentration, while the concentration of PaO2 and the value of BE were significantly $p \leq 0.001$ higher after surgical correction in comparison with the value before surgery. Moreover, males showed a significantly higher concentration of RBC, PCV, and hemoglobin than females after surgical correction. Conclusions: Surgery repair of (TOF) restore the arterial blood measurements PaCO2, PaO2, BE, RBC, PCV, and Hb concentration to the normal value in males and females.

KEY WORDS: Tetralogy of Fallot; PaO2; PaCO2; PCV; Surgical correction; Base Excess.
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1. INTRODUCTION:

The prevalence of congenital heart defects is eight in 1,000 births worldwide. TOF is the most common cyanotic congenital heart defect which represents 10% of all heart defects. The severity of cyanosis is determined by the degree of pulmonary blood flow obstruction (Wilson *et al.*, 2019). Tetralogy of Fallot prevalence varies from 0.26 to 0.48% for every 1000 live birth (Agarwalla, 2017). TOF is a congenital cardiac malformation consisting of interventricular contact, often referred to as a ventricular septal defect (VSD), right ventricular hypertrophy, right ventricular outflow tract obstruction, an override of the ventricular septum by the aortic root (Bailliard and Anderson, 2009).

Niels Stensen described it in 1672, Edward Sandiford in 1773, and the French physician Étienne-Louis Arthur Fallot in 1888, after whom it is named. Currently, patients typically present as neonates, with cyanosis of varying severity, depending on the degree of blockage of blood supply to the lungs. The etiology is multifactorial, but the associations identified include untreated maternal diabetes, phenylketonuria, and retinoic acid intake. The probability of familial recurrence is 3%. The chest radiograph, electrocardiogram, and echocardiogram are valuable medical measures for diagnosis. The echocardiogram provides a definitive diagnosis and typically provides appropriate details for the preparation of surgical care (Bailliard and Anderson, 2009).

More than 85% of infants with congenital heart disease are expected to reach adulthood, while the 40-year survival rate for those with TOF is at least 90% (Hickey *et al.*, 2009). When compared to younger patients with TOF surgical correction,

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patients over 35 years of age have a substantially higher mortality rate (Dorobantu *et al.*, 2020). If untreated, ventricular and atrial arrhythmias develop in up to 35% of TOF patients, and the rate of sudden heart death is 6% during a follow-up period of up to 30 years (Murphy *et al.*, 1993). Also, since many patients in early adulthood have no symptoms, they are frequently lost to follow-up, possibly losing the chance of treating problems until they become permanent (Mackie *et al.*, 2009).

The treatment has steadily progressed since the first surgical repair in 1954. Excellent long-term survival outcomes from the clinical methods commonly used in the treatment of TOF (30-year survival ranges from 68.5% to 90.5%). Depending on the level of right ventricular outflow tract stenosis and pulmonary artery anatomy, patients with TOF have different degrees of cyanosis. The anatomical anomalies were seen in TOF range from milder to more extreme phenotypes, such as pulmonary atresia and right ventricle double outlet type TOF. Different management and treatment strategies can involve these more extreme types (Van der Ven *et al.*, 2019).

The TOF contributes to low blood oxygenation. This is due to the mixing of oxygenated and deoxygenated blood through the ventricular septal defect in the left ventricle and the preferential flow of mixed blood through the aorta from both ventricles due to the restriction of flow through the pulmonary valve (Abdulla, 2011). Depending on the severity of the anatomical defects, blood oxygenation varies significantly from one patient to another. Symptoms range from no cyanosis or mild cyanosis to profound cyanosis at birth, depending on the degree of obstruction. It is often referred to as a 'pink tet' if the baby is not cyanotic. Other signs include a heart murmur that can range from almost imperceptible to very loud, trouble eating, weight loss, slow growth and physical development, exertional labored breathing (dyspnea), finger and toe clubbing, and polycythemia (Hay *et al.*, 2009).

The present study was done to evaluate the effect of TOF on some arterial blood measurements including pH, PaCO2, PaO2, BE, RBC, PCV, and Hb. Moreover the evaluation of the degree the repaired of these parameters to the normal level after surgery and successfully of the Fallot correction.

2.MATERIALS AND METHODS

2.1Subjects

The study included 80 persons and divided into two groups:

1- Males with TOF (40 patients).
2- Females with TOF (40 patients).

The study was carried out in the Cardiac Center in Erbil city from March /2018 to October/2020. The ages of the patients ranged from 3-33 years. The diagnosis of heart defect TOF) was based on clinical signs, echocardiography, and sometimes angiography for each patient.

2.2 Blood sample collection and analysis

Five ml of fresh blood obtained from each patient and put in a heparinized tube and the blood is added to the arterial blood gas analysis system (Medica, USA) for measurements of the pH, PaCO2, PaO2, and BE. Also, the RBC, PCV, and Hb concentration were measured by using a Coulter Counter instrument (Medonic, USA).

2.3 Statistical analysis

Analysis of data was performed by using Statistical Package for Social Science (SPSS), version 17. Results are expressed as mean ± S.E. Dependent t-test was used for comparison of different arterial blood parameters, RBC, PCV, and Hb concentration before and after surgery. A P-value of less than 0.05 was considered to be statistically significant.

3.RESULTS

Table 1 showed the effect of the TOF on arterial blood pH, PaCO2, PaO2, BE, RBC, PCV, and Hb concentration in male patients. The results showed that there is a significant $p \leq 0.001$ increase in the PaO2 95.05 ± 5.76 mmHg, Base excess -2.11 ± 0.34, mmol/L and a significant decrease $p \leq 0.001$ in the PaCO2 37.45 ± 1.74 mmHg, RBC 4.78 ± 0.10 $\times 10^6$/µL, PCV 40.26 ± 2.42 %, and Hb concentration 15.25 ± 0.34 g/dL in arterial blood after surgical correction when compared with the value before surgery PaO2 62.62 ± 5.97 mmHg, Base excess -4.28 ± 0.41, mmol/L, PaCO2 47.40 ± 2.22 mmHg, RBC 5.55 ± 0.12 $\times 10^6$/µL, PCV 50.66 ± 3.03%, and Hb 18.33 ± 0.41 g/dL .

The results presented in Table 2 showed the comparison of the arterial blood pH, PaCO2, PaO2, BE, RBC, PCV, and Hb concentration in female patients pre and postoperative correction. The results revealed that there is a significant $p \leq 0.001$ increase in the PaO2 93.99 ± 5.88mmHg,

Base excess -2.01 ± 0.29, mmol/L and a significant decrease p≤ 0.001 in the PaCO2 38.95 ± 1.89 mmHg, RBC 4.02 ± 0.14 $\times 10^6$/µL, PCV 36.33 ± 1.60%, and Hb concentration 13.43 ± 1.29 g/dL in arterial blood after surgical correction when compared with the value before surgery PaO2 56.80 ± 5.17 mmHg, Base excess -4.27 ± 0.51 mmol/L, pCO 48.22 ± 2.79 mmHg, RBC 4.86 ± 2.56 $\times 10^6$/µL, PCV 47.26 ± 2.56%, and Hb 17.23 ±1.51g/dL.

The comparison of pH, PaCO2, PaO2, Base excess, RBC, PCV, and Hb after postoperative correction between male and female TOF patients are observed in Table 3. The male patients showed a significantly p≤ 0.05 higher concentration of the RBC 4.78 ± 0.10 $\times 10^6$/µL, PCV 40.26 ± 2.42 %, and Hb 15.25 ± 0.34 g/dL than that of the females RBC 4.02 ± 0.14 $\times 10^6$/µL, PCV 36.33 ± 1.60%, and Hb 13.43 ± 1.29 g/dL after surgery. No significant differences were observed in the pH, PaCO2, PaO2, and BE between male and female patients after surgical correction.

Table 1. Arterial blood gases and blood parameters in pre and postoperative correction in male patients with TOF (Mean ± SEM)

Arterial blood gases and some blood parameters	Preoperative	Postoperative	p-value
pH	7.32 ± 0.01	7.39 ± 0.01	0.378
PaCO2 mmHg	47.40 ± 2.22	37.45 ± 1.74	0.001
PaO2 mmHg	62.62 ± 5.97	95.05 ± 5.76	0.001
Base excess mmol/L	-4.28 ± 0.41	-2.11 ± 0.34	0.001
Red blood cells $\times 10^6$/µL	5.55 ± 0.12	4.78 ± 0.10	0.001
Packed cell volume %	50.66 ± 3.03	40.26 ± 2.42	0.001
Hemoglobin g/dL	18.33 ± 0.41	15.25 ± 0.34	0.001
	N= 40		

P-value ≤ 0.05 considered significant N= number of samples
SEM = standard error of the mean

Table 2. Arterial blood gases and blood parameters in pre and postoperative correction in female patients with TOF (Mean ± SEM)

Arterial blood gases and some blood parameters	Preoperative	Postoperative	p-value
pH	7.34 ± 0.01	7.38 ± 0.01	0.378
PaCO2 mmHg	48.22 ± 2.79	38.95 ± 1.89	0.001
PaO2 mmHg	56.80 ± 5.17	93.99± 5.88	0.001
Base excess mmol/L	-4.27 ± 0.51	-2.01 ± 0.29	0.001
Red blood cells $\times 10^6/\mu L$	4.86 ± 2.56	4.02 ± 0.14	0.001
Packed cell volume %	47.26 ± 2.56	36.33 ± 1.60	0.001
Hemoglobin g/dL	17.23 ±1.51	13.43 ± 1.29	0.001
	N= 40		

P-value ≤ 0.05 considered significant N= number of samples

SEM = standard error of the mean

Table 3. Arterial blood gases and blood parameters comparison of postoperative correction between male and female patients with TOF (Mean ± SEM)

Arterial blood gases and some blood parameters	Males	Females	p-value
pH	7.39 ± 0.01	7.38 ± 0.01	0.378
PaCO2 mmHg	37.45 ± 1.74	38.95 ± 1.89	0.547
PaO2 mmHg	95.05 ± 5.76	93.99± 5.88	0.238
Base excess mmol/L	-2.11 ± 0.34	-2.01 ± 0.29	0.654
Red blood cells $\times 10^6/\mu L$	4.78 ± 0.10	4.02 ± 0.14	0.05
Packed cell volume %	40.26 ± 2.42	36.33 ± 1.60	0.05
Hemoglobin g/dL	15.25 ± 0.34	13.43 ± 1.29	0.05
	N= 40		

P-value $\leq$ 0.05 considered significant N= number of samples
SEM = standard error of the mean

4.DISCUSSION

The majority of children born with TOF undergo corrective surgery and live to adulthood (Long and Duffy, 1990). In recent eras, overall survival after TOF repair has improved significantly (Van der Ven *et al.*, 2019). The optimum age for full TOF surgical correction has fallen and is now considered to be between the ages of 3 and 12 months (Shinebourne *et al.*, 2006). The repair includes using ventricular septal defect patch closure and separating the aorta back to the left ventricle, resection of muscle bundles of the right ventricular outflow tract (RVOT), and decreasing the degree of valvular stenosis of RVOT (Sommer *et al.*, 2008).

In this study, the decrease in PaO2 and increase in PaCO2 in arterial blood in patients with TOF in both sexes is results in low blood oxygenation and due to the mixture of oxygenated and deoxygenated blood in the left ventricle via ventricular septal defect and preferential mixed blood flow. It appears that low preoperative oxygenation is one of the important factors influencing early mortality in TOF patients (Bhardwaj *et al.*, 2017). Owing to the obstruction of flow through the pulmonary valve, the mixed-blood flow from both ventricles through the aorta is preferential. This is known as a right-to-left shunt. The primary sign is low saturation of blood oxygen with or without cyanosis from birth or growth in the first year of life (Josos and Judas, 2020). The surgical correction returned PaO2 and PaCO2 to the normal level. In the present study, the Base excess decrease in the patients with TOF beyond the normal range in both sexes, and after postoperative correction, the values of Base excess returned to the normal value. The obtained results in the current study are in agreement with the findings of (Bhardwaj *et al.*, 2017), who found an increase in the PaO2 and a decrease in the Base excess after postoperative of patients with tetralogy of TOF in India.

Base excess and base deficiency in human physiology apply to an excess or deficit in the amount of base present in the blood respectively (Fischbach and Dunning, 2009). The additional base that must be applied to a liter of blood to normalize the pH is the base deficit. The substantial base deficit is a predictor of morbidity and mortality in patients who are seriously ill and trauma (Paladino *et al.*, 2008, Ouellet *et al.*, 2012). The value is generally stated as a concentration in mmol/L units, with positive numbers suggesting a base surplus and a deficit being negative. A standard BE comparison range is between −2 and +2 mmol/L. Comparing the base excess with the reference range helps decide whether a cardiac, metabolic, or mixed metabolic/respiratory problem triggers an acid/base disturbance. While the respiratory portion of the acid-base balance is characterized by carbon dioxide, the metabolic component is defined by BE. The measurement of BE is then specified under a standardized carbon dioxide pressure by titration back to a standardized blood pH of 7.40. Bicarbonate is the primary base that leads to base excess. A divergence of serum bicarbonate from the reference range is therefore typically expressed by a deviation in the BE (Fischbach and Dunning, 2009).

In our results, there is an increase in RBC, PCV, and Hb concentration. In most patients, the numbers of RBC increased above 6 $\times 10^6$/µl and causing secondary polycythemia. Congenital heart defects with right-to-left shunting are one of the causes of acquired polycythemia associated with hypoxia. Despite an increased Hb level, erythropoietin-mediated and hypoxia-dependent polycythemia is characterized by an elevated erythropoietin level (Sirhan *et al.*, 2005). In the study of (Gunduz *et al.*, 2014), the results found an increase in the RBC 7 × 10^6/µL, PCV 64.4%, Hg 21.5 g/dL in adult females with uncorrected TOF. A physiological reaction to tissue hypoxia is secondary erythrocytosis associated with cyanotic congenital heart disease, resulting in a rise in serum erythropoietin level, stimulating bone marrow erythropoiesis, resulting in increased red cell mass, PCV, and viscosity of the whole blood (Territo and Rosove, 1991, Warrell *et al.*, 2003). Increased oxygen-carrying capacity is provided by the rise in circulating red cells. This effect, however, is offset by the increase in the viscosity of serum that with consequent symptoms of hyperviscosity such as headache, visual disturbance, loss of concentration, paresthesia, muscle weakness, and fatigue, it decreases blood flow and tissue perfusion and impairs the delivery of tissue oxygen (Replogle *et al.*, 1967, Thorne, 1998).

Limitations

Included criteria

There are several limitations in this study. This is a retrospective cohort study with a small population, representing only one center. While the multiple centers may reflect a more diverse population, the type of surgery and timing were not standardized either within or across the institutions and may affect the ability to extrapolate results to other institutions. Additionally, Patients with intrauterine growth restriction have been noted to have alterations in fetal Doppler indices. This population was not excluded from this current study. The sample size of this retrospective cohort study was not thought to be large enough to adequately detect differences between groups with and without intrauterine growth restriction (IUGR).

Excluded criteria

Owing to the limitations of fetal imaging, I have removed patients who do not have a ductus arteriosus. Without the opportunity to run off, these people may have a very different physiology.. Other noncardiac factors that could have pushed an intervention forward or backward were not considered. Any births that were not carried to term or had their first cardiac operation after birth were excluded from the study. Patients who were removed from the sample due to chromosomal or neurologic anomalies would not benefit from these findings (which includes 22q11.2 deletion syndrome).

CONCLUSIONS

The current study concluded that TOF in both sexes caused a decrease in the arterial PaO2 and an increase in the PaCO2. Also, polycythemia with an increase in the number of RBC, Hematocrit, and Hb concentrations was observed in TOF patients. Operative correction restored the arterial blood gases, RBC, and Hb to the normal value.

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Conflict of Interest

The author declares no conflict of interest

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RESEARCH PAPER

Phytochemical screening and anti-candida activities of *Crocus cancellatus* herb. Ethanol extract

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A B S T R A C T:

The antifungal, antioxidant activity, total tannin estimation, and phytochemical screening of *Crocus Cancellatus* extract were investigated. The highest yield was obtained with ethanol solvent (36.1%). A significant amount of tannin was obtained in the plant (11.1%).

In Antifungal testing against *Candida* spp. The most effective Inhibitory obtained was 13.16mm against *C. Krusei*, while the less effective activity was 8.22 mm against *C. Glabrata*. The minimum inhibitory concentration (MIC) value obtained against all tested *Candida Spp*. candida strains within diameter rate of 12.5-25 µg/ml. The minimum inhibitory Effects of tested antifungal agents was FLU/25 which have no any inhibitory effects especially against was *Candida famata* While the highest inhibitory zone of FLU/25 38.40 mm was obtained against *Candida guilliermondii*. the maximum antioxidant activity obtained was 98.68% at 0.2 ml.

10 compounds were determined in the content determination study with LC-MS/MS. Gallic acid (46965.14 µg/g), Quinic acid (1935.71 µg/g), and malic acid (3831.37µg/g) were obtained at the highest levels. Vanillic acid (138.2 µg/g), Protocatechuic acid (116.87µg/g), tr-Ferulic acid (51.17µg/g), Quercetin (20.56 µg/g), p-coumaric acid (12.8 µg/g), Chlorogenic acid (2.51 µg/g) and Salicylic acid (1.56 µg/g) were obtained quantitatively less. These results confirmed that the *Crocus cancellatus* ethanol extract has potential antioxidant and antifungal activity

KEY WORDS:
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INTRODUCTION

The genus *Crocus* plant studied by many researchers since 1983. Till these days dozens of wild types were found in the areas, researched, and the consequence was published (Kerndorff and Pasche, 2004; Kerndorff and Pasche, 2006; Erol et al., 2011)**.** *Crocus cancellatus* genus had been used for many years in cooking to increase the sensorial standard of nutrition. Over the past few years, the purpose of nutrient flavour used in traditional cooking has admitted great care because of the growing concern in human health and studied both in vitro and in vivo by researchers (Loizzo et al., 2008; Iauk et al., 2015)**.** Crocus spice is a part of the Iridaceae family. The stigmas of the crocus should be collected by hand from the sensitive flowers upon opening to protect the attractive volatile ingredient (Ellis- Barrett, 2005)**.** With its unique bitter tasty, lightly and sharp like straw flavour, it has been used as a flavour and colourants in the nutrient (Moraga et al., 2009)**.** Attention and the effect of crocus on people's health are expanding because of its demonstrated health features (Mykhailenko et al., 2019)**.**

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Crocus can not only use as a garden flower but also used significant traditional remedy and cooking plant as well. Some result shows that crocus plant extract has antitumor, antimutagenic, cytotoxic activities, and inhibitory effects on intracellular nucleic acid and protein synthesis in malignant cells (Abdullaev et al., 2003**;** Chryssanthi et al., 2007**;** Tavakkol-Afshari et al., 2008)**.** *Crocus cancellatus* is a member of the Crocus genus that is an extremely common plant in Iran, Turkey, Jordan, and Greece (Erol et al., 2014)**.** The crocus genus has been used for antimicrobial, anti-fungal, antiseptic, and is also used as an antibacterial agent, antiseptic, and antiflatulent (Mykhailenko et al., 2019)**.** Because of the significance of these spices that it could be beneficial for treating this kind of pathogenic fungi disease. Tha aim of this researchs determind of total yield and antioxidant activity. The characterization of isolated phytochemicals compounds by using LC-MS/MS. Finaly, compare plant antifungals extracts with some artificial antifungal by antifungal activity by MIC and disc diffusion method. No, the previous study has been reported before.

MATERIALS AND METHODS

Materials

Preparation of extract

Plants bulb of *Crocus cancellatus* herb. was collected from Qarachugh maountain. Further, taxonomic identification was done at the herbarium at the Department of Biology College of Sciences Salahaddin University - Erbil. Plants identified. Then, the plant samples were saved at the of the Biology Department Herbarium. Finally, it was given the herbarium code for species started 7471 code (Carranza-Rojas et al., 2017). The extraction and analysis were done in Turkey at DICILA university. Ten grams of plant powder were put into the Erlenmeyer flasks and 100 ml solvent was added. The plant and solvent ratio was 1:10. The Microwave milestone NEOS system equipped with vessels and an automatic temperature control system was used for the microwave-assisted extractions (MAE). This method of analyze has been done by Microwave method was used for 60 minutes until the boiling point temperature. Afterwards, to get pure extract the mixture was filtered and evaporated with a rotary evaporator (Chan et al., 2011).

Methods

Our study has been consisting of four analysis extraction, extract yield percentage determination, determination of total condensed tannin and DPPH radical scavenging activity. It has been done at Kahramanmaraş Sütçü İmam Üniversitesi laboratory. Also, phytochemical analyses were done at Dicla University in Turkey.

Calculation of extraction yield

The extraction yield is a calculation of solvent efficiency to extract specific components from the original material and it was described as the amount of extract recycle in bulk comparison according to the initial quantity of dry sample (Ali Abu-Mejdad, 2014**;** Ismael et al., 2019)**.**

About 0.5-gram plant powder processed with 50 ml of ethanol solvent. The extract yield percentage was calculated as the following formula:

$$Yield\ percentage\ (\%) = X/Y\ _{x}100$$

$$Where:\ X\ is\ the\ oven\ dry\ weight\ of\ extract\ (g), Y\ is\ the\ dry\ weight\ of\ the\ sample\ (g)$$

Determination of total condensed tannins

The 0.05 gram $FeSO_4$, 95 ml C_4H_9OH, and 5 ml HCl were prepared. For the analyzed condensed tannin, 0.01gram of root powder and mimosa tannin placed one by one in a tube and a 10 ml solution was added then heated for an hour in the water bath. The analyzed was measured by UV/Vis at 580 nm (Ismael et al., 2019).

Antioxidant activity

The plant extract of free radical scavenging activity of root was calculated by the DPPH method (Rahman et al., 2017). Firstly, 0.1mM DPPH was prepared for the extraction process. Afterwards, 0.1, 0.2, and 0.3 ml of solutions completed with a solvent until 3 ml respectively. Then, 1 ml of DPPH was added. After that, experiment tubes were vortexed and all was kept in a dark place for 30 minutes. The absorbance of the samples carried out by a UV/vis

spectrophotometer at 517 nm. BHT (Butylated hydroxytoluene) was used as a reference. calculations were done according to the following formula;

$$\textbf{DPPH radical scavening activity } (\%) = \frac{\boldsymbol{Abs_{control} - Abs_{test}}}{\boldsymbol{Abs_{control}}} \boldsymbol{x\ 100}$$

Where: $\boldsymbol{Abs_{control}}$ = the absorbance of DPPH radical + solvent
$\boldsymbol{Abs_{test}}$ = the absorbance of the sample and BHT, separately

Phytochemical Screening

MS instrumentation

MS detection was achieved using a Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer prepared with an ESI source operating in both positive and negative ionization modes. LC-MS/MS data were collected and processed by Lab Solutions software (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used to quantify the analyses: the assay of investigated compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and/or the third one for confirmation(Ertas et al., 2015).

Method validation parameters for LC-MS/MS

In this investigation, twenty-four phenolic and three non-phenolic organic acids which were prevalent in plant resources were quantified and qualified in plants. Straight-lined regression equations and the linearity ranges of the investigated standard compounds are assumed in Figure 1 (Gülçin et al., 2003; Ismael et al., 2019). Correlation coefficients were originating to be upper than 0.99. The limit of detection (LOD) and limit of quantitation (LOQ) of their ported analytical method were shown with results in Table 5. For the analyzed compounds, LOD ranged between 0.05 and 25.8 g/L and LOQ ranged between 0.17 and 85.9 g/L. Additionally, the recoveries of the phenolic compounds ranged from 96.9% to 106.2%. The results were calculated by the equation below:

$$\textbf{Quantification } (\mu\textbf{g analyte/g extract}) = \frac{\mathbf{Y} * \mathbf{U_{95}}}{\mathbf{100}}$$

Where; **Y**: LC-MC/MS result of an analyte, **U**: uncertainty confidence level

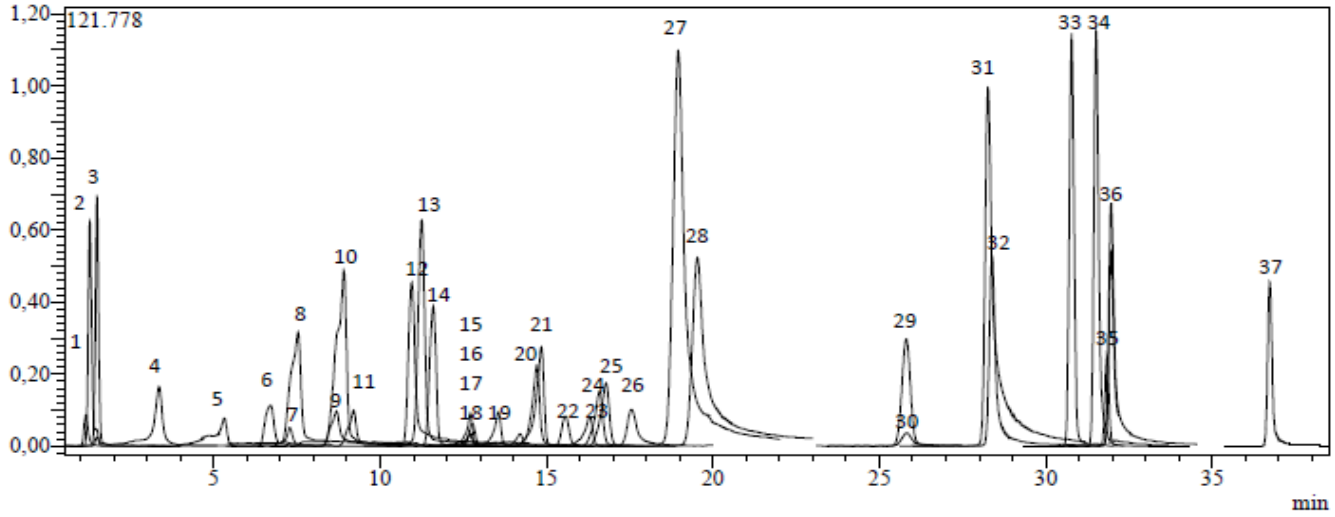

Figure 1 LC-MS/MS chromatogram for reference phytochemical compounds

The tested Candida

All Candida spp. in the present study was obtained from (Media Diagnostic Centre) MDC Erbil accredited from America, CAP* (College of American Pathology), *Candida albicans* (ATCC 10231), *Candida glabrata, Candida Krusei, Candida tropicalis, Candida famata, Candida parapsilosis, and Candida guilliermondii*. To extend the spectrum of organisms studied, local isolate were also involved in the examination. Admittedly, six selected antifungal discs used Nystatin 100unit (NYS), Clotrimazole 10mcg CLT, Fluconazole 25mcg (FLU), Ketoconazole 10mcg (KTZ), Fluconazole 1010mcg (FLC) and Miconazole 10mcg (MCZ) for comparison with plant antifungal (50 µg/ml).

Biological assesses

The Disc diffusion method was known as the Kirby- Bauer method was particularly standardized by the Clinical Laboratory Standard Institute (CLSI 2006) methodology. Suspensions of the tested *Candida* spp. were prepared in sterilized saline the optical density (OD) of a 0.5 McFarland standard using a densitometer (Turbidity meter BioMerieux, France) (Dalynn Biologicals et al., 2007) A purified About 0.1 ml of prepared Candida suspension was inoculated into SDA medium then spread by using sterilized cotton swab was immersed in the suspension and inoculated evenly over the entire superficies of the medium by swab three times over the entire agar surface; rotate the plate (Z Sahin et al., 2019). The plant extract was prepared by dissolving 0.1 g of the extract with 100 mL of their respective solvents. The discs were 6 mm in diameter include a (50µl) concentration of extracts. Which was applied to the plates only by applying sterile forceps and then smoothly pushed down on the agar medium. Normally, no more than 6 disks are placed on a plate for avoids overlapping of the zones of inhibition and potential failure in the analysis. Later, for more acceptance by sterile forceps were used to place the antifungal disc on the surface of the inoculums. After the disks were located on the plate for each *Candida* sp. six disc plant extract, the plate was modified and incubated at 37°C (Air incubator, LTE Scientific, UK) for 48h dependent on strains being tested. Next, the inhibition zone was measured At that moment, it was determined the inhibition zone by using an a ruler. Finally, all inhibition zone described chart table which is confirmed by NCCLS for antimicrobial susceptibility to examination and monitoring (National Committee for Clinical Laboratory Standards, 2015).

The minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) rates achieved by broth microdilution, crocus extract activity against *Candida* spp. was comparatively low. That placed 0.9 ml of Sabouraud dextrose broth (SDB) medium. Afterwards, 0.1 ml of plant extract at concentration 0.1 mg/ml solvent was stirred with broth in following decimal dilutions diluting 1 ml in 9 ml of broth to obtain the concentration range of 0.1 to 500 mcg/ml. The inoculum could be standardized based on optical density [OD625 of 0.08-0.1(1cm light path)] analyzed by spectrophotometer. This is normally done after 18- 24 h. All the candida suspensions were arranged by suspending 24h candida culture in sterile normal saline (0.89% NaCl wt/vol). The turbidity of the candida suspension was regulated according to the 0.5 McFarland standard (equivalent to 1.5×108 CFU/ml) (Costa et al., 2014; Rahman et al., 2017)

Statistical analysis

The data examined by ANOVA using GraphPad (Prism 6) statistical program. A statistical analysis Probability value (P-value) (<0.05) was accepted as statistically meaningful (*), while the P-value than (>0.05) was regarded as statistically not meaningful. Histogram and pie-chart were accepted for the statistical analysis of the consequences (Hedges and Rhoads, 2010; Rahman et al., 2017).

RESULTS AND DISCUSSION

Determination of yield

The *Crocus cancellatus* extract was extracted by a microwave method. The highest extraction yield was obtained by ethanol solvent (36.1%) was given in Table 1.

It has been determined in previous studies that the yield of the crocus family is generally high. In the study conducted with three different solvents (hexane, dichloromethane, and ethanol) of the genus *C. sativa*, it was determined that the best yield was obtained by the ethanol extract (Wali et al., 2020).

Table 1. Determination of *C. cancellatus* extract yield

	R 1	R 2	R 3	Yield (%)
Mean	36.2 ±	35.8 ±	36.3 ±	36.1 ±
SD	0.009	0.008	0.007	0.008
SE	0.003	0.002	0.003	0.0025

R: Replication

Determination of tannin

Tannin was measured by the n-butanol-HCl-iron method. The result of the tannin is given in Table 2. This method was used as a standard for n-butanol/HCl assay is mimosa-tannin under normal reaction/condition which measured using the regression equation ($y = 66.357x + 0.4117$) got previous from the linear calibration curve. Measuring the high quantity of tannins detected in all crocus extract by the percentage of tannin was determined.

In our experiment, tannin-content was diverse certainly and meaningfully therefore the result was displayed as an absorbance unit at 580 nm per 1 mg of extract (A580/mg). The result was viewed (11.1 mg/kg) as shown in Table 2.

Table 2. Total condensed tannin

	R 1	R 2	R 3	Mean
Extract (mg/kg)	10.6 ±	11.4 ±	11.3 ±	11.1 ±
SD	0.12	0.13	0.12	0.1
SE	0.18	0.16	0.18	0.18

SD: Stander Division; SE: Stander Error

$$\boldsymbol{Calculation}: \% = \boldsymbol{A/3m}$$

Where A = absorbance value, m = mass weight [26].

Antifungal analysis

The Crocus plant extracts inhibition zones' results were considerably different tested *Candida* spp. The results are represented in Table 3. The minimum inhibitory zone was obtained against *Candida Krusei* (8.22 mm). Whereas, the highest inhibitory zone was obtained against *Candida glabatra* (13.16 mm). Furthermore, the minimum inhibitory concentration (MIC) results against all Candida species were obtained among 12.5 - 25 µg/ml represented in Table 3. The various artificial antifungal was statically meaningful by the various widths of inhibition zones of maximum the activities against the organisms examined were given in Table 3. The mean inhibition zones artificial antifungal against Candida species experiment was obtained between 0–38.4 mm. The minimum inhibitory zone 0 mm of FLU/25 was obtained against *Candida famata* Whereas the highest inhibitory zone of FLU/25 38.40 mm was obtained against *Candida guilliermondii.* In the previous research, methanol extract of *C. Sativus* at different concentrations (4500 µg/well, 9000 µg/well, and 13500 µg/ well) show different antimicrobial results against bacteria. The result shows that *C. sativus* extract was indicated that the plant has the resource of some substances which has an antimicrobial effect against microorganism. especially, against Listeria spp. Moreover, as the extract concentration increased, the antimicrobial effect also increased

(Muzaffar et al., 2016; Jadouali et al., 2018). Petal extracts of C. Sativus showed a strong effect at the minimum concentration against pathogenic bacteria. Both *P.aeruginosa* and *S.aureus* gave some results that they inhibited at the lowest inhibitory concentration of 15.63 µg/mL. Petals extracts were not much more impact in inhibiting the growth of *E. coli* and *C. albicans* at the lowest concentration. However, it showed high concentration antimicrobial and antifungal effects (Muzaffar et al., 2016; Wali et al., 2020).

Table 3. Inhibition zones (mm) and MIC of the extracts and synthetic antifungal activities against the *Candida spp*.

Candida spp.		Inhibition	MIC	FLU	MCZ	FLU	KTC	NY	CLT
		Zone	(µg/ml)	10mcg	10mcg	25mcg	10mcg	100 U	10mcg
Candida albicans	Mean	11.3	250	6	14.61	10.55	14.05	20.21	18.15
	(mm)	±	±	±	±	±	±	±	±
	SD	0.1	0.05	0	0.1	0.13	0.1	0.24	0.18
	SE	0.05	0.03	0	0.04	0.05	0.04	0.1	0.07
Candida glabatra	Mean	9.17	250	16.71	14.54	15.68	13.33	24.72	12.29
	(mm)	±	±	±	±	±	±	±	±
	SD	0.011	0.63	0.16	0.2	0.21	0.11	0.31	0.22
	SE	0.08	0.41	0.06	0.08	0.09	0.04	0.12	0.09
Candida krusei	Mean	8.22	250	15.32	13.52	24.63	18.79	14.49	25.91
	(mm)	±	±	±	±	±	±	±	±
	SD	0.03	0.02	0.15	0.2	0.66	0.09	0.17	0.22
	SE	0.018	0.017	0.06	0.08	0.27	0.04	0.07	0.09
Candida tropicalis	Mean	8.63	250	20.52	12.46	27.67	23.96	21.23	19.95
	(mm)	±	±	±	±	±	±	±	±
	SD	0.017	0.18	0.08	0.27	0.17	0.1	0.15	0.14
	SE	0.1	0.08	0.03	0.11	0.07	0.04	0.06	0.06
Candida famata	Mean	13.16	125	6	6	6	6	24.75	20.26
	(mm)	±	±	±	±	±	±	±	±
	SD	0.012	0.55	0	0	0	0	0.13	0.16
	SE	0.10	0.34	0	0	0	0	0.05	0.07
Candida parapsilosis	Mean	12.23	125	21.43	11.53	26.36	27.48	24.47	22.42
	(mm)	±	±	±	±	±	±	±	±
	SD	0.022	0.024	0.16	0.17	0.17	0.32	0.15	0.28
	SE	0.013	0.022	0.07	0.07	0.07	0.13	0.06	0.11
Candida guilliermondii	Mean	11.8	250	37.48	24.19	38.4	37.61	21.45	31.26
	(mm)	±	±	±	±	±	±	±	±
	SD	0.63	0.13	0.26	0.15	0.42	0.32	0.18	0.19
	SE	0.21	0.01	0.11	0.06	0.17	0.13	0.07	0.08

Antioxidant activity

The crocus plant extract exhibited a DPPH radical scavenging activity. The maximum antioxidant activity at 0.2 mL was (98.68%). While the

minimum quantity was at 0.1 mL (96.60%). The results are represented in Table 4. The results show that the plant extracts had better antioxidants activity than the BHT as control.

As a result of the antioxidant activity study with *Crocus sativus*; it was determined that the plant extract had approximately the same results compared to α-tocopherol (Jadouali et al., 2018). In another study with *C. sativus*, it was reported that the antioxidant results obtained were similar to the previous studies, and the antioxidant activities of the leaves were less than stigma but higher than corm with increasing concentration (Ismael et al., 2019; Wali et al., 2020). In our antioxidant activity study with *C. cancellatus* species; the activity of all concentrations of the root extract was higher than the control (BHT).

Table 4. DPPH Radical Scavenging activity (%)

	MWE 0.1ml	MWE 0.2ml	MWE 0.3ml	BHT 0.1ml	BHT 0.2ml	BHT 0.3ml
Mean (%)	96.60 ±	98.67 ±	97.85 ±	69.9 ±	68.10 ±	72.7 ±
SD	0.13	0.18	0.21	0.23	0.28	0.35
SE	0.08	0.09	0.08	0.11	0.12	0.15

BHT (synthetic antioxidant), MWE: Microwave extraction

Analytical parameters

In this study, the phenolic acids in Crocus plant extract were investigated by UHPLC-ESI-MS/MS. Various 10 phytochemicals in ethanol extract were identified. Thirty-seven non-phenolic, phenolic, and flavonoid compounds of root ethanol extract had been investigated. Analytical parameters and results are given in Table 5 and Figure 2 . As shown in the table high quantity of gallic acid (46965.14µg/g), Quinic acid (1935.71µg/g), and malic acid (3831.37µg/g) were obtained. Moreover, the lowest amount of vanillic acid (138.2µg/g), Protocatechuic acid (116.87µg/g), tr-Ferulic acid (51.17µg/g), Quercetin (20.56µg/g), p-coumaric acid (12.8µg/g), Chlorogenic acid (2.51µg/g) and Salicylic acid (1.56µg/g) were detected. Investigated the chemical composition of Crocus sativus by HPLC; In the structure of the plant, Kaempferol, Quercetin, and Myricetin flavonoids and phenolic acids such as Caffeic acid, Chlorogenic acid, and Gallic acid were determined, However, Genistein and Cumaric acid have not been detected in its structure (Gismondi et al., 2012). Compounds that support previous studies in our plant structure have been identified and these compounds are known to have antioxidant effects.

A lot of research has been done and reported on the importance of tannin and its variation. Their activity is possible due to their ability to bind with extracellular and soluble proteins or associate with the cell wall of fungi. The character of these compounds can disrupt fungal membranes (Hasmida et al., 2014). The radical scavenging activity mechanism of root extracts may be related to the formation of polyphenolic compounds. It has previously been shown that polyphenolic compounds are responsible for the radical scavenging activity, as they impart hydrogen atoms to active free radicals (Kheirandish et al., 2016). The variance in amount levels of phenolic compounds could be due to differing methods of extraction (Garzón et al., 2009). Indeed, it could be due to the polyphenolic content of the roots being greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall, altitude and hightide, soil nutrients. etc.(Manach et al., 2004).

Table 5. Analytical parameters that belong to the LC-MS/MS method

	No Analytes	RT[a]	Mother ion (m/z)[b]	Fragment ions	Ion. mode	Equation	R^{2c}	RSD%[d] Interday	Intraday	Linearity Range (µg/L)	LOD/LOQ (µg/L)[c]	Recovery (%) Interday	Intraday	U[f]
1	Quinic acid	1.13	190.95	85.3-93.3	Neg	y=41.06×+10671	0.996	0.00259	0.00274	250-10000	75.8/79.4	1.00288	0.98778	1935.71
2	Malic acid	1.23	133.00	115.2-71.3	Neg	y=316.95×-42041	0.999	0.00477	0.00527	250-10000	55.3/67.5	1.01266	0.99836	3831.37
3	Fumaric acid	1.48	115.00	71.4	Neg	y=64.99×-11592	0.997	0.00536	0.00460	100-5000	28.1/34.5	0.99748	0.99867	N.D
4	Gallic acid	3.00	168.85	125.2-79.2	Neg	y=226.76×+38152	0.998	0.01601	0.01443	250-10000	95.5/106.9	1.00004	1.00454	46965.1
5	Protocatechuic acid	4.93	152.95	108.3	Neg	y=297.75×+30590	0.995	0.01236	0.01296	100-5000	28.2/31.4	0.99404	1.01070	116.87
6	Pyrocatechol	6.48	109.00	108.35-91.3	Neg	y=30.61×+14735	0.996	0.01313	0.01339	1000-20000	261.1/278.4	0.99987	0.99936	N.D
7	Chlorogenic acid	7.13	353.15	191.2	Neg	y=781.36×-18697	0.998	0.00058	0.00076	25-1000	6.2/8.1	1.00806	0.99965	2.51
8	4-OH-benzoic acid	7.39	136.95	93.3-65.3	Neg	y=409.03×+112079	0.998	0.01284	0.01538	250-10000	33.2/38.1	0.99662	1.00058	N.D
9	Vanillic acid	8.57	166.90	152.3-108.3	Neg	y=35.84×-12097	0.999	0.00528	0.00619	1000-20000	122.2/139.7	1.00093	1.04095	138.2
10	Caffeic acid	8.80	178.95	135.2-134.3	Neg	y=3963.32×+178156	0.998	0.01454	0.01469	25-1000	18.4/22.4	1.00917	0.98826	N.D
11	Syringic acid	9.02	196.95	182.2-167.3	Neg	y=42.33×-52547	0.996	0.01049	0.01345	1000-20000	212.5/233.3	0.99922	0.99977	N.D
12	Vanillin	10.87	151.00	136.3-92.2	Neg	y=446.10×+70934	0.998	0.00696	0.00793	250-10000	44.3/53.1	0.99679	0.99611	N.D
13	Salicylic acid	11.16	136.95	93.3-65.3	Neg	y=5286.26×+309192	0.989	0.01016	0.01242	25-1000	5.0/6.5	1.00989	0.99013	1.56
14	*p*-Coumaric acid	11.53	162.95	119.3-93.3	Neg	y=3199.20×+13002	0.992	0.01820	0.01727	25-1000	7.3/9.1	1.00617	1.01224	N.D
15	Rutin	12.61	609.05	300.1-271.1	Neg	y=561.91×-16879	0.997	0.00473	0.00624	25-1000	5.5/6.5	1.00994	0.98017	N.D
16	Ferulic acid	12.62	192.95	178.3	Neg	y=80.45×-31782	0.997	0.00708	0.00619	250-10000	36.6/42.0	0.99987	1.00289	51.17
17	Sinapinic acid	12.66	222.95	208.3-149.2	Neg	y=141.96×-73294	0.992	0.01446	0.01517	250-10000	78.7/86.1	1.00164	0.99962	N.D
18	Hesperidin	12.67	610.90	303.1-465.1	Poz	y=1340.27×-43769	0.998	0.00945	0.01126	25-1000	3.4/4.2	1.01733	1.01263	N.D
19	Isoquercitrin	13.42	463.00	300.1-271.1	Neg	y=803.23×+4981	0,999	0.00682	0.00515	25-1000	5.4/6.3	1.00594	1.00722	N.D
20	Rosmarinic acid	14.54	359.00	161.2-197.2	Neg	y=909.67×-201692	0.994	0.02014	0.01751	100-5000	6.6/8.8	0.99206	1.03431	N.D
21	Nicotiflorin	14.68	593.05	285.1-255.2	Neg	y=498.38×+79274	0,991	0.00737	0.00875	100-5000	22.4/25.5	1.02558	1.00970	N.D
22	*o*-Coumaric acid	15.45	162.95	119.4-93.3	Neg	y=1219.34×-10915	0.999	0.02730	0.02566	25-1000	24.4/31.1	0.98344	0.99061	12.8
23	Rhoifolin	16.11	577.05	269.2-211.1	Neg	y=237.15×+11887	0,999	0.00747	0.01528	100-5000	23.1/27.9	1.01046	1.01739	N.D
24	Quercitrin	16.41	447.15	301.1-255.1	Neg	y=339.39×+38910	0.999	0.01528	0.02320	100-5000	22.0/25.2	0.99726	1.00620	N.D
25	Apigetrin	16.59	431.00	268.2-239.2	Neg	y=1775.55×+91121	0,993	0.01797	0.01607	25-1000	5.4/6.1	1.01394	1.00419	N.D
26	Coumarin	17.40	147.05	91.0-103.2	Poz	y=33.64×-89700	0.994	0.01306	0.01239	1000-20000	208.4/228.4	0.99947	1.00081	N.D
27	Myricetin	18.72	317.00	179.2-151.3	Neg	y=583.55×+205727	0,999	0.00652	0.00711	250-10000	53.2/57.2	0.99982	1.00042	N.D
28	Fisetin	19.30	284.95	135.2-121.3	Neg	y=547.46×+274791	0,991	0.00557	0.00820	250-10000	54.4/61.4	0.99877	1.00031	N.D
29	Cinnamic acid	25.61	147.00	103.15-77.3	Neg	y=9.06×-12403	0.996	0.00648	0.00816	5000-20000	821.8/859.7	1.00051	0.99927	N.D
30	Liquiritigenin	25.62	254.95	119.3-135.1	Neg	y=2384.96×+59141	0,996	0.01849	0.01738	25-1000	5.5/6.6	1.00333	0.99957	N.D
31	Quercetin	28.17	300.90	151.2-179.2	Neg	y=1198.48×+480562	0.990	0.01589	0.01360	100-5000	23.3/28.9	0.98470	1.00103	20.56
32	Luteolin	28.27	284.75	133.2-151.2	Neg	y=3272.65×+150557	0,997	0.00575	0.00696	25-1000	5.4/6.5	1.00772	0.99524	N.D
33	Naringenin	30.68	270.95	151.2-119.3	Neg	y=4315.1×+178410	0.995	0.02054	0.02019	25-1000	5.4/6.4	0.99883	1.01002	N.D
34	Apigenin	31.43	268.95	117.3-151.2	Neg	y=4548.36×+295252	0.990	0.02304	0.02204	25-1000	5.4/6.3	1.01444	1.01331	N.D
35	Hesperetin	31.76	300.95	164.2-136.2	Neg	y=876.67×+48916	0.997	0.03209	0.02605	25-1000	5.6/6.9	0.98850	0.99435	N.D
36	Kaempferol	31.88	284.75	255.1-117.3	Neg	y=26.29×+87558	0,992	0.01436	0.01070	1000-20000	206.6/214.3	0.99971	0.99851	N.D
37	Chrysin	36.65	252.95	143.3-119.4	Neg	y=2032.13×+95593	0,993	0.00490	0.00630	25-1000	5.4/6.2	1.00338	1.00437	N.D

([a]RT: Retention time, [b]Mother ion(m/z): Molecular ions of the standard compounds (m/z ratio), [c]R2: Coefficient of determination, [d]RSD: Relative standard deviation, [e]LOD/LOQ (µg/L): Limit of detection/quantification, [f]U (%): percent relative uncertainty at 95% confidence level (k=2)

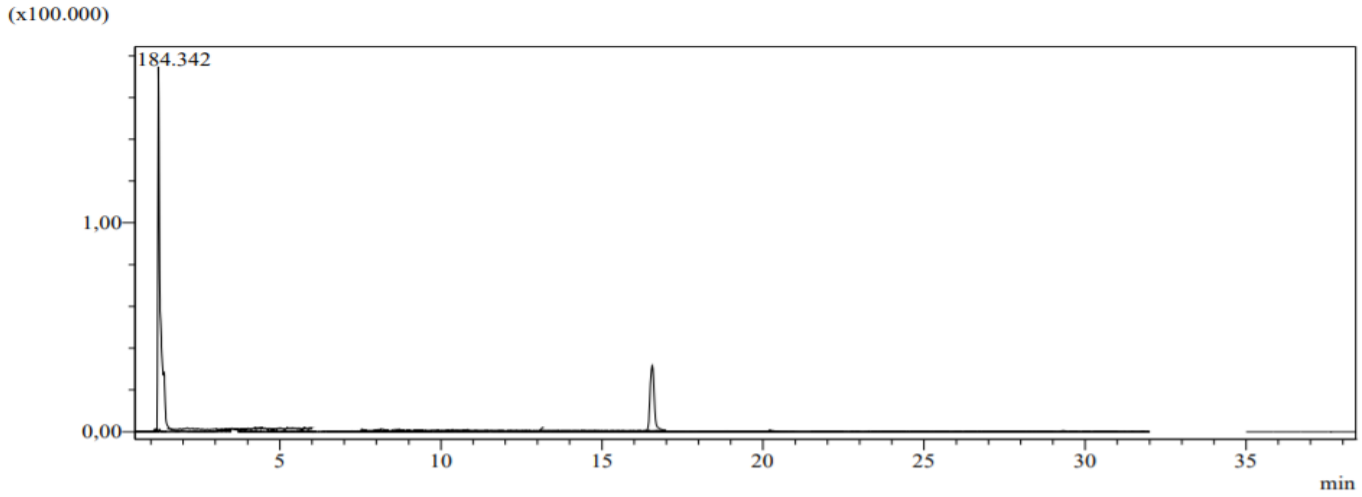

Figure 2 :LC-MS/MS chromatogram for analytical parameters and results of phytochemical compounds

Conclusion

This research examined the phytochemical screening and the potency biological activity of *Crocus Cancellatus* on seven pathogenic *Candida* spp. Based on the results, *C. cancellatus* plant extract has given good yield and tannin result, as well as plant extract, has strong antifungal effective on fungi which used for the experiment and also has a strong antioxidant effect because of the high levels of phenols and flavonoids. Gallic acid, Malic acid and Quinic acid were identified as the main compounds. This plant extract can be used as a natural additive in the chemical, cosmetic, and food industries due to its ingredients.

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