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RESEARCH PAPER

# Demonstration of tissue and peripheral blood eosinophils in patients with acute appendicitis

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**A B S T R A C T:**

Acute appendicitis (AA) is the most surgical emergencies. Clinical diagnosis is usually confirmed by adjuvant laboratory testing but histopathology is the gold standard for diagnosis. Acute eosinophilic appendicitis (AEA) is a rare form of appendicitis with muscularis propria infiltration and muscle fiber edema. This study was aimed to find simple and accurate way for the diagnosis of unusual eosinophilic appendicitis that may requires postoperative special treatment. A prospective investigation was conducted at Shahid Dr. Khalid Teaching Hospital in (Koya city/ Kurdistan Region/ Iraq), This involves randomly selecting fifty appendectomy samples from emergency department patients from November 2021 to May 2022. Acute appendicitis is suspected in these patients based on their medical history, physical examination, investigation, and abdominal ultrasound. The patients were 28 (56% males) and 22 (44% females). Patients ranged in age from 4 to 43 years, with a mean of 20.98 years, and The majority of cases (60%) are under 20. A significant gradual (increase in weight and decrease in dimensions) of appendix with different age group was seen. Theoretical and actual means of CBC parameters differed significantly, although there was no significant sex difference. Tissue and peripheral blood eosinophil counts were directly correlated ($P < 0.01$), although there was a negative correlation between tissue and WBC count ($P<0.0019$). Congo red was statistically highly significant ($P< 0.0001$) in detecting tissue eosinophils compared to MGG and H&E. In conclusion, Congo red better than H&E and MGG staining for identifying tissue eosinophils, also tissue and peripheral blood eosinophils were positively correlated.

KEY WORDS: Acute Appendicitis (AA), Acute Eosinophil Appendicitis (AEA), histopathological and Congo red.
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## 1. INTRODUCTION:

A tiny finger-like blind-ended tube called the vermiform appendix connects to the cecum (Faisal *et al*., 2022) Which is arise from posteromedial wall of caecum about 2cm below the ileocecal junction and has no constant position (Iqbal *et al*., 2018, Alraddadi, 2021). Base of appendix has constant relationship with caecum, but the location of the tip was changeable (Iqbal *et al*., 2012, Gali *et al*., 2022).

The length of a normal appendix varies between 6 to 9 cm (Ekici *et al*., 2018) as well as it might range between 2 to 20 cm (Schumpelick *et al*., 2000, Deshmukh *et al*., 2014). Histologically the appendiceal wall consists of 4 layers and has several unique features and specialized cells (Iurii *et al*., 2018). innermost columnar-lined mucosa with enterocytes, specialized cells like goblet cells, entero-endocrine cells, and Paneth cells, surrounded by submucosa with an externally located muscularis externa and serosa (Bandyopadhyay *et al*., 2022). The presence of masses of lymphoid tissue in the mucosa and submucosa of the appendix is the distinguishing feature of this anatomical structure (Bharti *et al*., 2016, Pawlina and Ross, 2018, Salih *et al*., 2020).

unavailable or decreases to a low concentration (Puchalska and Crawford, 2017).

Acute appendicitis is the medical term for the acute inflammation of the appendix (AA) and the majority of abdominal surgical emergencies occur worldwide (Teng *et al*., 2021). The severity of

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acute appendicitis may range from mild acute appendicitis to fecal peritonitis (Sartelli *et al*., 2018, Mekakas *et al*., 2022). Males have an 8.6% lifetime chance of developing acute appendicitis, while women have 6.7%. Most instances occur in people between 10 and 20 (Snyder *et al*., 2018). Acute appendicitis developed as a result of a luminal obstruction, which causes distention and an increase in pressure inside the lumen. This increased intraluminal pressure then caused the appendix's ischemia and mucosal hypoxia to develop, as well as ulceration, a breach in the mucous barrier, and the onset of necrosis (Sazhin *et al*., 2021). Fecalith, lymphoid hyperplasia, foreign bodies, and tumors are the most typical causes of obstruction. Appendicitis is also brought on by infectious pathogens such viral, bacterial, and parasite infections that induce inflammation of the local lymphatic tissues (Podany *et al*., 2017, Shahmoradi *et al*., 2021).

Clinical diagnosis is mainly based on a patient's medical history, physical findings, lab tests, and hospital imaging, but a histopathological analysis is still the gold standard for confirmation of diagnosis (Téoule *et al*., 2020). Vulnerable periumbilical pain, nausea, vomiting, anorexia, pain spreading to the right lower quadrant, and low-grade fever are all common symptoms of appendicitis. The most frequent kind of therapy is still laparoscopic appendectomy. However, mounting data shows that in around 70% of patients, broad-spectrum antibiotics, such as piperacillin-tazobactam monotherapy or combination treatment with either cephalosporins or fluroquinolones and metronidazole, effectively cure acute appendicitis that is not complicated (Moris *et al*., 2021). Several inflammatory cells involvement within the appendectomy specimens with the clinical diagnosis of appendicis is proved. The mixed inflammatory cells like neutrophils, mature lymphocytes and eosinophils are observed within the different layers of the appendix confirming the final diagnosis as "appendicis. (Kafle *et al*., 2020). Eosinophils are normal constituents of the appendix in the lamina propria and submucosa, but not in the muscularis propria. Finding of a pure eosinophil infiltrate amidst inflammatory edema in the muscle layer makes one suspect allergy as a cause for the acute onset disease (Ahn and Lee, 2021).

Eosinophil counts in all the layers were very high in acute eosinophilic appendicitis compared to normal appendices (Mowla, 2021). A rare variation of appendicular inflammation that may be related to allergy or parasitic infection is known as acute eosinophilic appendicitis (AEA). It is described as the absence of neutrophils and the presence of eosinophils in the muscular layer of the appendix as observed in acute suppurative appendicitis. The presence of edema separating the muscle fibers and eosinophilic infiltration of the muscularis propria on histopathology is the gold standard for the diagnosis (Aggelidou *et al*., 2019). In addition, eosinophils appeared more sensitive than neutrophils in correlation with early acute clinical symptoms for appendicitis, implying that increased eosinophils in muscularis propria may be a marker for early symptomatic appendicitis (Zhang and Qu, 2022).

## 2. MATERIALS AND METHODS

### 2.1 Chemical substances

In this study, distilled water, Ethanol, formalin, Xylene, Paraffin, DPX, Methanol, sodium hydroxide, H&E, MGG and Congo red are used.

### 2.2 Experiment design

A prospective investigation was conducted at Shahid Dr. Khalid teaching Hospital in Koya city/ Kurdistan Region/ Iraq, during the period of six months from 1st Nov. 2021 to 1st May. 2022. The study was approved by the ethical committee in department of biology which included a randomized collection of fifty appendectomy samples from patients admitted to emergency department. According to the history, physical examination, investigation, and abdominal ultrasound, there is a clinical suspicion of acute appendicitis.

Clinical information includes age, gender, pain intensity, duration, kind, point of starting, and shifting. All patients had preoperative abdominal ultrasounds and had blood and urine samples submitted for examination. All patients were treated by an open appendectomy.

Preoperatively blood samples were collected in EDTA tubes from all patients. EDTA anticoagulated blood used to measure complete blood count CBC (WBC, RDW and platelet parameters), and for peripheral blood eosinophils in blood film. Eosinophils were counted per 200 WBC by using high power field (1000 x).

After appendectomy all samples were grossly examined for weight, dimension &photographed immediately fixed in 10% neutral buffered formalin and at the base, center, and tip, three cross sections were obtained. Sections were routinely processed, paraffin embedded and stained by routine H and E stain (Bancroft and Stevens,1982). All samples were further stained by two stains May Grunwald- Giemsa (MGG) (Atom Scientific) and special Congo red stain (Sigma Aldrich).

Eosinophils were counted in the (mucosa and submucosa) layers of all three sections. From both layers (10) fields were selected from each section for eosinophils counting, But Best oriented and stain sections not necessary in adjust fields. The average eosinophil count for each case obtained and expressed as number of cells per high-power field.

### 2.3 Data Analysis, Statistical Analysis:

The results were expressed as Mean ± Mean Standard Error (MSE) and analyzed statistically using one-way analysis of variance ANOVA. Calculations were done by Graph Pad Prism software version 9. The students t-test was used for analyzing the data between two groups. Ordinary one- way ANOVA was used for analyzing the data between three age groups. The one way repeated measures analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for comparisons between stains. One sample t test used for analyzing the actual means and theoretical means of CBC parameters. The results were considered significant at $P \leq 0.05$. Furthermore, Pearson correlation coefficient (r) was used find the relationships between the tissue eosinophil (age, gender, appendix dimension and peripheral blood eosinophil).

## 3. RESULTS

This prospective study included fifty (50) cases with preoperatively clinical diagnosis of acute appendicitis and treated by open appendectomy. Among their patients, twenty-eight (28) were men (56%), and twenty-two (22) were women (44%).

### 3.1 Age distribution in acute appendicitis

This research revealed that the patients' ages ranged from 4 to 43 years (20.98 ± 1.217) and majority of the patients (30 cases) (60%) were between (4-20) years. Postoperative appendicular dimensions and weight correlated with age of the patients. Table (1).

**Table 1:** Weight and dimensions of appendix in different age groups.

| Age groups / years | High (cm) Mean ± SE | Length (cm) Mein ± SE | Width (cm) Mean ± se | Weight (gm) Mean ± se |
|---|---|---|---|---|
| ≤ 20 (n = 30 ) | 0.6645 ± 0.04920 | 7.231 ± 0.2924 | 1.257 ± 0.1424 | 3.904 ± 0.2810 |
| 21– 30 (n = 12) | 0.5000 ± 0.02981 ## | 5.629 ± 0.6707 * | 0.9333 ± 0.1364 | 5.829 ± 0.6632 * |
| ≥ 30 (n = 8) | 1.029 ± 0.2407 * | 5.420 ± 0.4375 ** | 0.7500 ± 0.1190 | 6.771 ± 1.077 ** |

Values were expressed as mean ± SE. asteroid represent the significant change between all the groups that compared with ≤2years age. Asterix represent the significant change between 20 - 30 age that compared ≥30 age.

#### 3.1.1 Weight of appendix

Postoperative fresh appendicular weight ranged from 1.2 to 11.5 gram (5.126 ± 0.371) The weight of the appendix was significantly ($P<0.0437$) lower in patients ≤ 20years age group when compared with (21-30 years) age group and those ≥30years age group ($P<0.0019$). However, there was a non-significant difference between 21-30 years and ≥ 30years' patient samples. Figure (1).

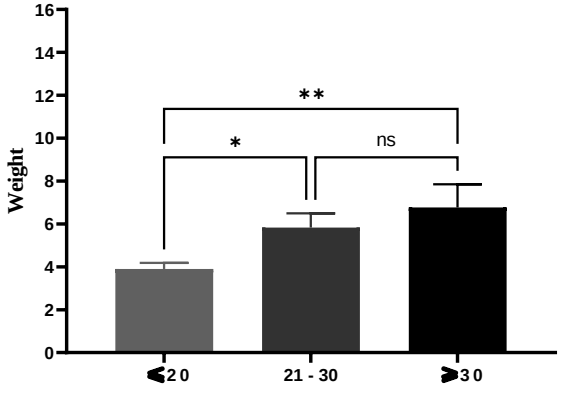



**Figure (1):** Appendicular weight in the study age groups.
Asterix means significant
NS means non-significant
Weight/gram
Age ≤ 20…… ≥ 30

#### 3.1.2 Length of appendix

Postoperative fresh appendicular length ranged from 2 to 13 cm (6.824 ± 0.270). The length of the appendix was significantly ($P<0.0429$) higher in patients ≤20years age group when compared with

(21-30years) age group and those ≥30years age group (P<0.0465). However, there was a non-significant difference between 21-30 years and ≥ 30 years. Figure (2).

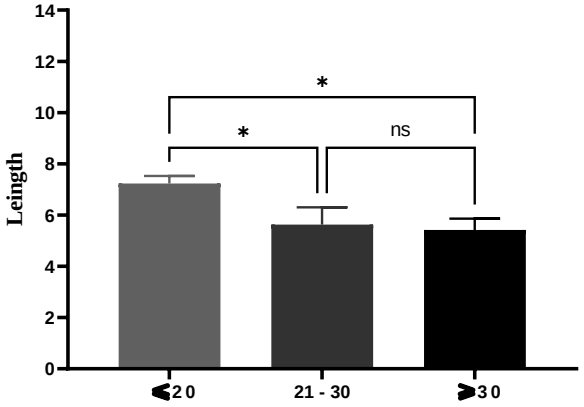



**Figure (2):** Appendicular length in the study age groups.
Asterix means significant
NS means Non-significant
Length/cm
Age ≤ 20……≥ 30

### 3.1.3 Width of appendix

The results showed that postoperatively width of appendix was non-significantly increased between all age groups that illustrated in figure (3).

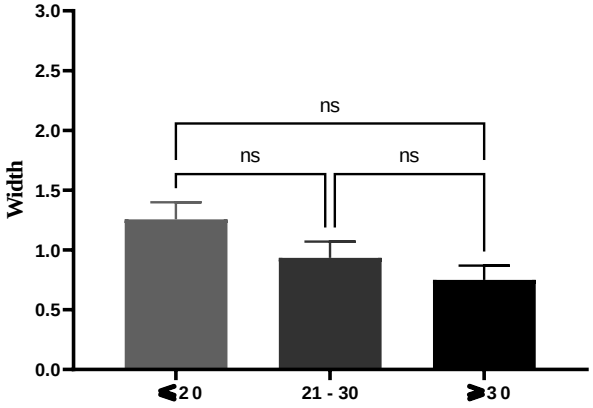



**Figure (3):** Appendicular width in the study age groups.
NS means non-significant
Width /cm
Age ≤ 20…… ≥ 30

### 3.1.4 Height of appendix

Postoperative fresh appendicular height ranged from 0.3 to 2.4 cm (0.7 ± 0.052). The height of the appendix was non-significantly higher in patients ≤ 20years age group when compared with (21-30years) age group. But, significantly was (P<0.0276) lower when compared with ≥30 age group, however, there was significant (P<0.0053) decrease between 21-30 and ≥ 30 groups. Figure (4).

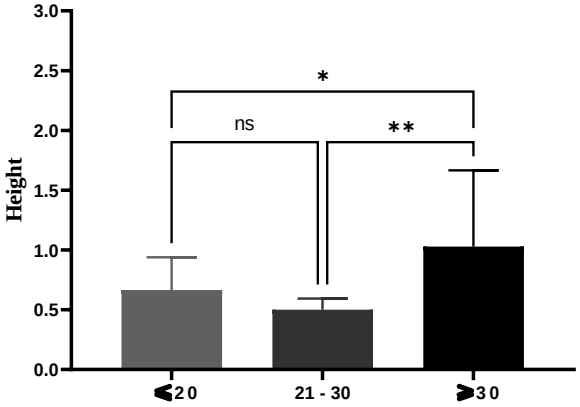



**Figure (4):** Appendicular height in the study age groups.
Asterix means significant
NS means Non-significant
height/cm
Age ≤ 20……≥ 30

## 3.2 Blood parameters in acute appendicitis

The current study showed that male patients were more than females (n=28) and (n=22) respectively. However, the blood parameters had a statistically non -significant differences between male & females as seen in table (2).

**Table 2:** Gender distribution and mean values of complete blood count parameters in acute appendicitis.

| Variable | Male (n = 28) Mean ± SE | Female (n = 22) Mean ± SE | P value |
|---|---|---|---|
| **WBC ($10^3$/µL)** | 11.85 ± 0.7413 | 11.31 ± 0.9012 | NS |
| **Neutrophil ($10^3$/µL)** | 7.068 ± 0.8763 | 8.732 ± 0.8364 | NS |
| **Eosinophil ($10^3$/µL)** | 2.089 ± 0.3054 | 1.318 ± 0.2042 | NS |
| **Lymphocyte ($10^3$/µL)** | 2.289 ± 0.1640 | 2.350 ± 0.2074 | NS |
| **Platelet ($10^3$/µL)** | 264.1 ± 13.09 | 295 ± 14.53 | NS |
| **MPV fL** | 7.786 ± 0.1224 | 7.755 ± 0.1408 | NS |

But there was statistically significant difference between actual and theoretical means of peripheral blood parameters. Table (3)

**Table 3:** Theoretical and actual means of CBC parameters.

| Variable | Theoretcal mean | Actual mean | SEM | P value |
|---|---|---|---|---|
| **WBC (103/µL)** | 13.35 | 11.62 | 0.5694 | 0.0037 |
| **Neutrophil (103/µL)** | 13.16 | 7.800 | 0.6187 | 0.0001 |
| **Lymphocyte (103/µL)** | 2.760 | 2.316 | 0.1282 | 0.0011 |
| **Platelet (103/µL)** | 315.6 | 277.9 | 9.882 | 0.0004 |
| **MPV fL** | 5.78 | 7.772 | 0.0914 | 0.0001 |
| **N/L %** | 5.65 | 3.385 | 0.1987 | 0.014 |
| **RDWC %** | 14.17 | 13.09 | 0.1917 | 0.0001 |

The actual means of WBC, lymphocytes and N/L were significantly decreased (P<0.0037), (P<0.0011), (P<0.014) respectively in comparison with theoretical means. However, the actual means of neutrophils, platelets and RDW were highly significantly decreased (P<0.0001), (P<0.0004), (P<0.0001) respectively compared with theoretical means. But the actual mean of MPV was highly significantly (P<0.0001) increased compared with theoretical mean.

### 3.3 Demonstration of eosinophils

Histologic demonstration of eosinophils in all postoperatively tissue sections were varied according to the stains used in this study.

There was highly significant difference between stains as illustrated in figure (5), but there was non-significant deference in tissue eosinophils between male and female according to stains figure (6).

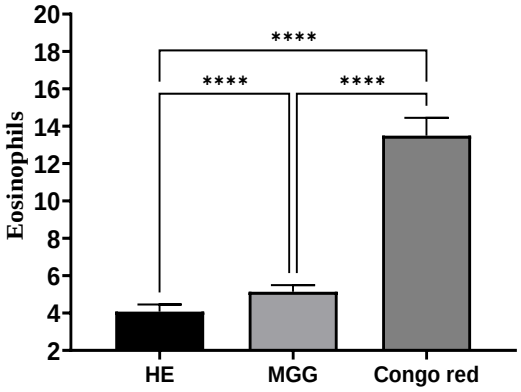



**Figure (5):** The histogram showed the highly significantly differences in tissue eosinophils count according to H&E, MGG and Congo red.

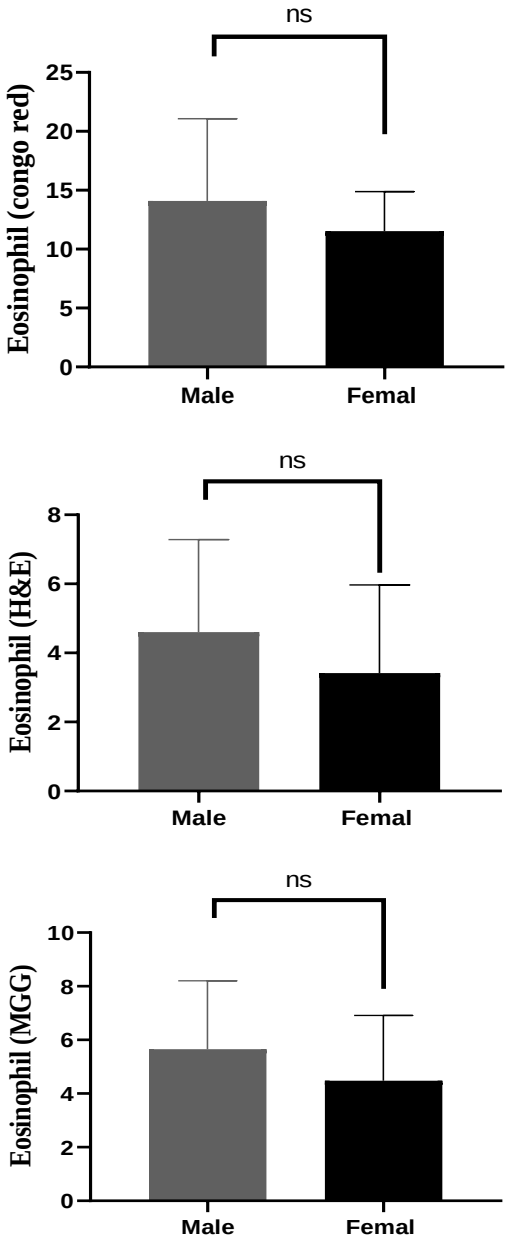



**Figure (6)**: The histogram showed the non-significantly differences in tissue eosinophils count by H&E, MGG and Congo red according to the sex.

Tissue sections stained by Congo red 3.50 ± 0.9540) revealed that tissue eosinophil count was

highly significantly (P < 0.0001) increased when compared with H&E (4.082 ± 0.3769) and MGG (5.136 ± 0.3602) Table (4).

**Table 4:** Demonstration of eosinophil by H&E, MGG and Congo red

| Name of stains | Eosinophil count in tissue Mean ± SE |
|---|---|
| **Hematoxylin & eosin** | 4.082 ± 0.3769 |
| **MGG** | 5.136 ± 0.3602 **** #### |
| **Congo red** | 13.50 ± 0.9540**** |

Values were expressed as mean ± SE. asteroid stain. Hash represents the significant change represent the significant change between all the groups that compared with hematoxylin and eosin between MGG stain that compared Congo red stain.

### 3.4 Correlations of tissue eosinophil

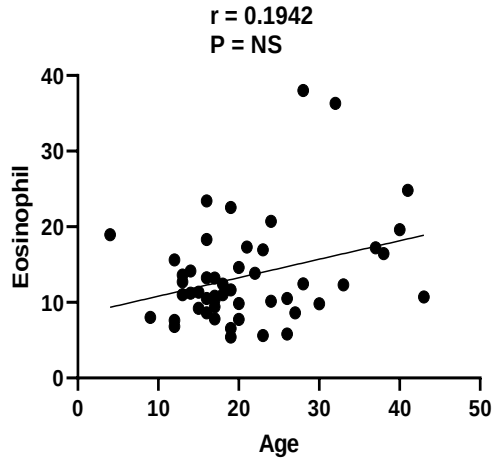
There was a correlation (using spearman correlation test) between tissue eosinophil as dependent variable when compared with age, sex, weight and dimension of appendices as independent variable in patients with acute appendicitis. Table (5).

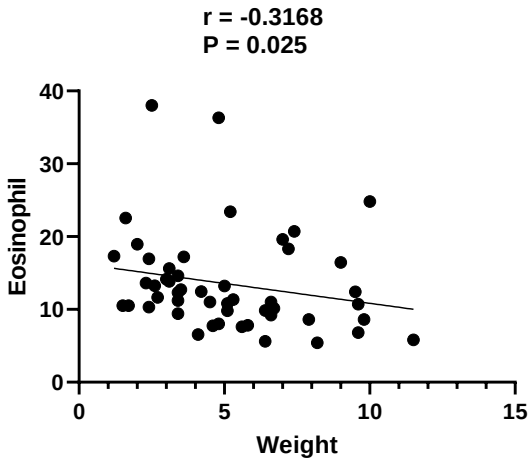
**Table 5:** Correlation between tissue eosinophil with the age, sex, weight and dimension.

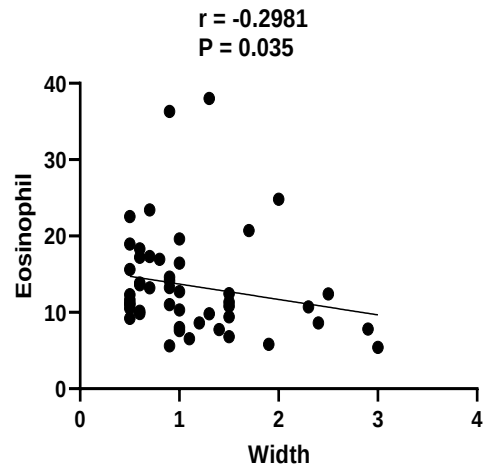
| Variable | r | CI | P value |
|---|---|---|---|
| **Age (years)** | 0.1942 | - 0.09730 to 0.4551 | 0.1765 |
| **Weight (gm)** | -0.3168 | - 0.5528 to 0.03369 | 0.0025 |
| **Length (cm)** | -0.043 | -0.3251 to 0.2462 | 0.7669 |
| **Width (cm)** | -0.2981 | - 0.53883 to 0.0131 | 0.0035 |
| **Height (cm)** | -0.2398 | - 0.4922 to 0.04975 | 0.0093 |
| **WBC ($10^3$/µL)** | -0.4289 | - 0.6368 to 0.1627 | 0.0019 |
| **Blood Eosinophil ($10^3$/µL)** | 0.3452 | 0.6557 to 0.5746 | 0.0141 |

(r) Means correlation coefficient, (CI) means confidence interval and Age between ≤ 20……. ≥ 30

Between tissue eosinophil and peripheral blood eosinophil count, a significant positive correlation was discovered. (p = 0.0141). Positive, Age and tissue eosinophil did not significantly correlate with one another (p = 0.1765), but a negative non-significant correlation was detected between tissue eosinophil and (sex and length) (p = 0.5436 and p = 0.7669) respectively. However, there was negative highly significant correlation between tissue eosinophil and gross appendicular parameters (weight, width, height and total WBC) (p = 0.0025, p = 0.0035 and p =0.0093) (p = 0.0141) respectively. As seen in Figure (7).



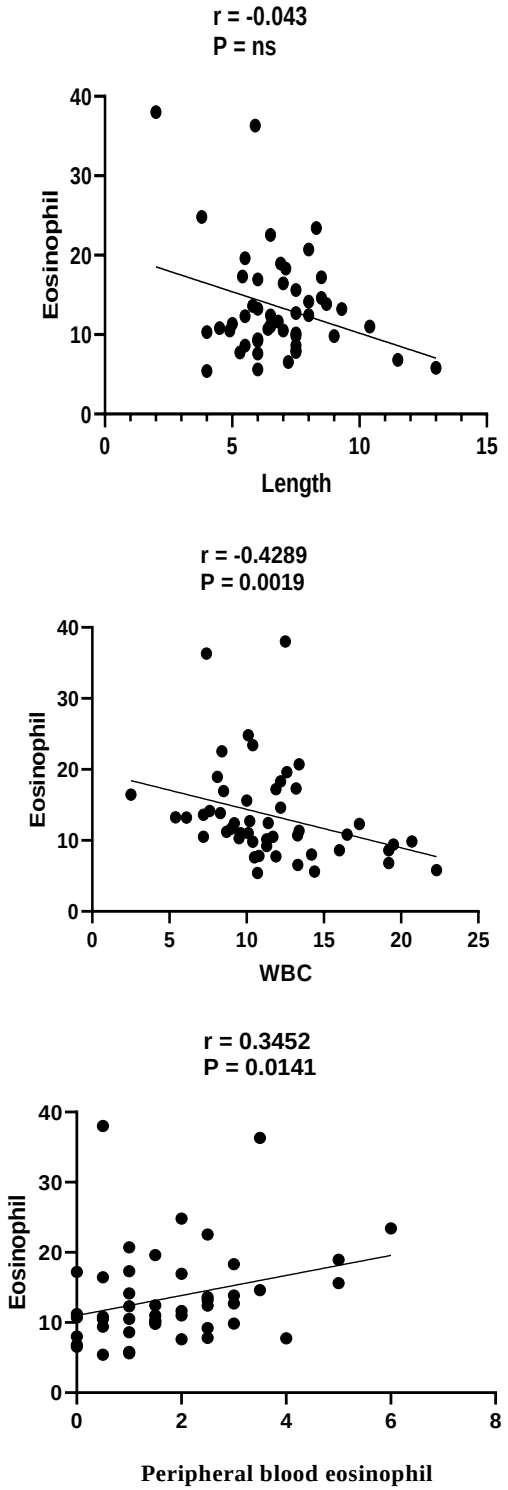



**Figure (7):** Scatter plot for the study of the relationship between Tissue eosinophil with Age, Sex, WBC, Peripheral blood eosinophil, weigh and Dimension. The statistical analysis was carried out with Spearman correlation test.

## 4. DISCUSSION

The most prevalent kind of surgical emergency in the world is acute appendicitis, which is defined by the development of inflammation at the local level, followed by a more broad inflammatory response (Dixon and Singh, 2020). The majority of acute appendicitis cases, despite some persistent controversy, are thought to be caused by luminal obstruction, which results in distention and increased pressure within the lumen, increased intraluminal pressure, appendix ischemia and mucosal hypoxia, ulceration, a breach of the mucous barrier, and the development of necrosis (Sazhin *et al*., 2021). Furthermore, (Garza-Serna *et al*., 2016) showed that the development of AA may also be influenced by environmental, regional, nutritional, genetic predisposition, and infections factors. Moreover, (Aggelidou *et al*., 2019) proposed allergic etiology for acute appendicitis.

In the current research, 50 patients in total were examined, and the findings revealed that men were affected more than females, with 28 (56%), and 22 (44%), patients, respectively. Our finding agrees with (Oguntola *et al*., 2010) who found an increase in the frequency of appendicitis in men; this may be attributable to differences in male and female body physiology. This was also verified by a different research, which found that the male ratio was higher than the female ratio (Ohene-Yeboah and Abantanga, 2009, Memon *et al*., 2013, Danish, 2022).

Appendicitis incidence is significantly influenced by age (Al-Mulhim, 2011). It is uncommon in newborns, but it increases in frequency throughout childhood and the early years of adulthood, peaking in the adolescent and early 20s. The likelihood of getting appendicitis decreases after middle age (Williams *et al*., 2008). Typically, it closely resembles lymphoid development (Akbulut *et al*., 2011). In the current research, the incidence was found to be greater 30 (60%) among patients whose ages ranged from (≤20) years. It has been believed that the high incidence of the disease is caused by the peak in lymphoid tissue development that occurs around adolescence, which increases the appendix's capacity to block. This was in line with studies (Kolur *et al*., 2014) which showed a greatest number of occurrences during the first and second decades of life, with a decline in incidence after the third decade. Furthermore (Rahman *et al*., 2008) was reported that in 60% of cases, appendicitis was caused by lymphoid hyperplasia that resulted in occlusion of the appendix's interior, indicating that the appendix is usually subject to abnormal lymphoid tissue proliferation. The findings of these studies are comparable to those of a study conducted in Sulaimani City, Iraq by (O.Ahmed, 2006).

The existing research showed that the weights and dimensions of appendices varied significantly depending on the age groups. The weight of appendix was higher in group (≥ 30) than group (21– 30), (6.7 gm) and (5.8gm) respectively, so there was a gradual increased with age groups and this was agreeing with study conducted by (Mohammadi *et al*., 2017) in which the highest weights of appendix was (7.04gm) in group (30-39) and lowest was (2.65gm) in group (< 10). But in contrast (Salih *et al*., 2020) was reported that the highest weights of appendix were (7.64gm) in group (20-29) and lowest was (4.13gm) in group (60-69) because of the individual differences in immune response strength and the probable decline in the number of lymphatic follicles, their replacement by connective tissue.

Knowledge of variation in appendiceal dimension is important during appendectomy, the association between age and appendiceal dimensions was statistically changes in existing study the length, width and height was significantly decreased gradually with age. The length of appendix was more in age group (≤ 20). probably the appendix achieves its adult sizes after an initial development phase that lasts from early infancy to around three years and does not continue to grow throughout childhood (Searle *et al*., 2013), This result was comparable to that of another research done by (Patel and Naik, 2016) According to their findings, the vermiform appendix is longer in young adults and children, and as people age, it progressively becomes shorter. Furthermore, our findings were similar with the results of (Paul *et al*., 2011). But in contrast (Ghorbani *et al*., 2014) found that the length of the appendix is longer in elderly people. Also there are some different findings in other study (Salih *et al*., 2020) which was reported that the vermiform appendix's length gradually reduced with age and that males' vermiform appendixes were somewhat longer than females'.

Delay in treatment of acute appendicitis may causes perforation. Therefore, achieving the correct diagnosis is crucial to decrease the chance of perforation and negative appendectomy (Andersson, 2007). In addition to the history, physical exam, and radiographic studies, inflammatory indicators are helpful in determining the diagnosis of appendicitis. Yet they can't be employed by themselves because of their limited sensitivity and specificity (Ahmed *et al*., 2019).

In this study blood parameters had a statistically non -significant differences between male & females, but there was statically significant difference between actual and theoretical means of CBC parameters. the actual means of WBC, lymphocytes, neutrophils and platelets were significantly decreased (P<0.0037), (P<0.0011), (P<0.0001), (P<0.0004) respectively in comparison with theoretical means. However, the actual means of WBC, neutrophils and lymphocytes were less than the theoretical mean, but Increased WBC and neutrophil count was the early marker of inflammation in acute appendicitis and may aid in acute appendicitis diagnosis. Although a decreased lymphocyte count was strongly correlated with clinical diagnosis of acute appendicitis. This discovery matched to that seen in other study conducted by (Ulukent *et al*., 2016, Al-Jawdah and Kamal, 2022) which reported that the WBC and neutrophil counts were significantly higher but lymphocytes were significantly lower in acute appendicitis.

Although the actual means of N/L and RDW were significantly (P<0.014), (P<0.0001) decreased compared with theoretical means. A helpful biomarker for the diagnosis of AA is elevated N/L this was agree with (Al-Jawdah and Kamal, 2022, Jung *et al*., 2017). But RDW is not useful as a biomarker for AA and this was agreeing with study conducted by (Anand *et al*., 2022). In contrast RDW indices have been reported for identifying acute and perforated appendicitis (Haghi *et al*., 2020). Also (Tartar *et al*., 2020) reported a high level of RDW in complicated cases.

The function and activation of platelets are related to mean platelet volume. The actual mean of MPV was highly significantly (P<0.0001) increased compared with theoretical mean, in the diagnosis of acute appendicitis, MPV level was not a reliable indicator and this computable with (Akbulut *et al*., 2019, Dooki *et al*., 2022). In contrast with (Tanrikulu *et al*., 2014, Tullavardhana *et al*., 2021) they reported decreased MPV in acute appendicitis patients. Presence of inflammatory cells such as eosinophil within the wall of the appendix is required for histological diagnosis of AEA which diagnosed by edema infiltration of muscularis layer by eosinophils, so the gold standard for the diagnosis of AEA is still histopathology analysis and these can be demonstrated by different routine & special stains (Kinoshita *et al*., 2019).

Hematoxylin and eosin (H&E) stains are a common, simple, affordable, and reliable

procedure that are used in many labs (AbdEl-Latif *et al*., 2016). H&E stain cytoplasm of eosinophils deep red (figure 8) due to their granular, but Background tissue stained with eosin reduces the ability to distinguish eosinophil granules visually and reduces the optical contrast between the target cells and the background. In this study, histologic demonstration of eosinophil varied according to H&E when compared with MGG and Congo red. the results revealed that H&E was highly significantly (P<0.0001) decreased in comparison with MGG and Congo red and this was matched with other study (Ikeda *et al*., 2022) As compared to H&E staining, the number of counted eosinophils was greater with Congo red and Giemsa. Moreover, (Meyerholz *et al*., 2009) reported that eosinophil identification with H&E may be successful. The "overlapping" staining pattern and morphology of eosinophils and neutrophils, however, might provide challenges for certain researchers. Although (Joshi and Kaijkar, 2013) matched the result of present study which showed that eosinophils are often simple to identify in standard H&E sections, occasionally these granulocytes take on an unusual form, particularly in fibrous tissue and inflammatory infiltrate, making their diagnosis in routinely stained sections quite challenging.

Due to its simplicity and consistency, MGG seems to be preferable than H&E in that it stained eosinophil granules pink (figure 9) with less background staining of other tissue structures (Lee and Kim, 2015). Giemsa staining was better than H&E staining in terms of making eosinophilic granulocytes visible under a microscope because they were more distinct (Lin *et al*., 2005). Despite the background staining and poor staining intensity but the yield of Giemsa as a diagnostic tool was significant as compared to H&E (p= 0.0001). Another study conducted by (Samoszuk, 1997) reported that Giemsa staining usually makes the intensely red granules of eosinophils considerably easier to see within tumor tissue.

Because of their special ability to bond with eosinophils, however, specialized stains like Congo red have also shown to be an effective diagnostic tool for identifying eosinophils. It is particularly important in acute appendicitis However, is one of the simplest, most affordable, and most accurate procedures available (Jain *et al*., 2014). In this study Congo red staining was quite effective at demonstrating eosinophils (P = 0.0001) as compared to H&E and MGG staining and the results were statistically highly significant. A cytoplasm that was brightly dyed red made eosinophils easy to identify (figure 10). Even (Sujatha *et al*., 2022) reported that histochemical analysis of Tumor Associated Tissue Eosinophilia (TATE) in Oral Squamous Cell Carcinoma using Congo red can help highlight the eosinophils for better identification and counting. Furthermore, (Song *et al*., 2018) also reported that for more accurate eosinophil counts in nasal polyps, Congo red and Chromotrope 2R are both more suitable due to their specificity and reproducibility. In contrast (Debta *et al*., 2012, Debta *et al*., 2010) reported for showing the presence of tissue eosinophils in tumor stroma, carbol chromotrope stain is preferable than Congo red.

In our study from the comparison of eosinophils in particular layer (mucosa and submucosa) of the appendectomy specimen with (age, sex, dimension, WBC and peripheral blood eosinophils) we found that there is non-significant correlation between eosinophils infiltration and (age, sex and length). However, a negative (Inverse) correlation between eosinophil infiltration and (weight, width, height and WBC) found which is statistically significant (0.0025), (0.0035), (0.0093) and (0.0019) respectively, but there was a positive (Direct) correlation between eosinophil infiltration and peripheral blood eosinophil which is statistically significant with (P < 0.01) and this was matched with other study (Carvalho *et al*., 2022).This positive (Direct) correlation means as there is increase in the number of eosinophils in tissue, there is also increase in the number of peripheral blood eosinophils.

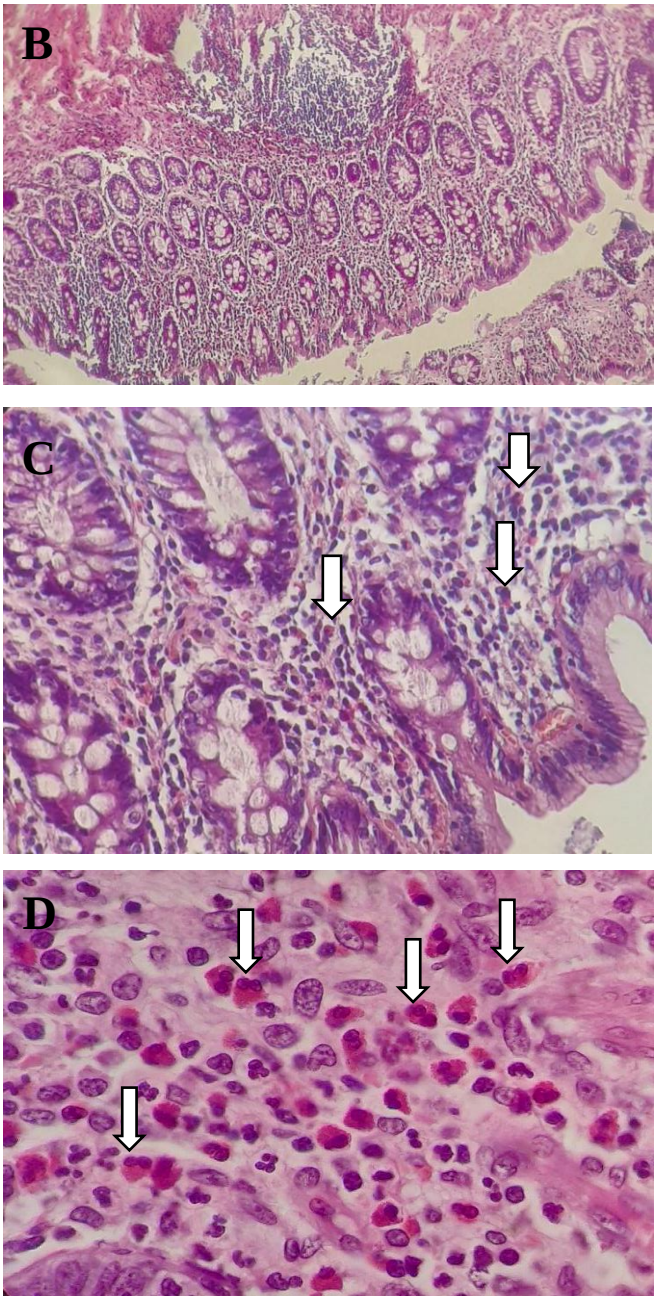
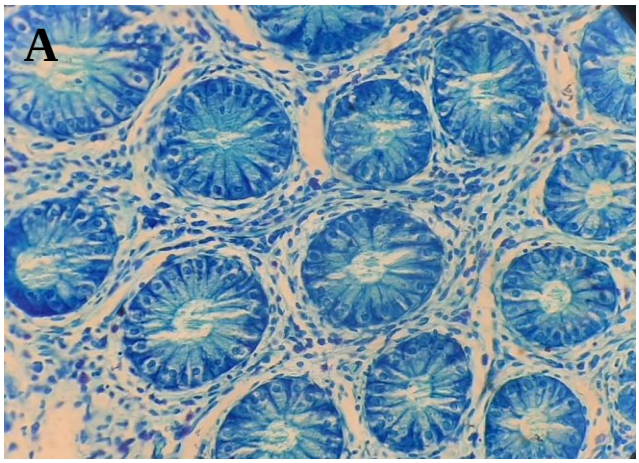



**Figure (8):** Appendicitis with increased number of eosinophils (arrows) infiltrating the lamina propria layer. (A) Cross section 40X, (B) Cross section 100X (C) Cross section 400X, (D) Cross section 1000X (H&E) stains.



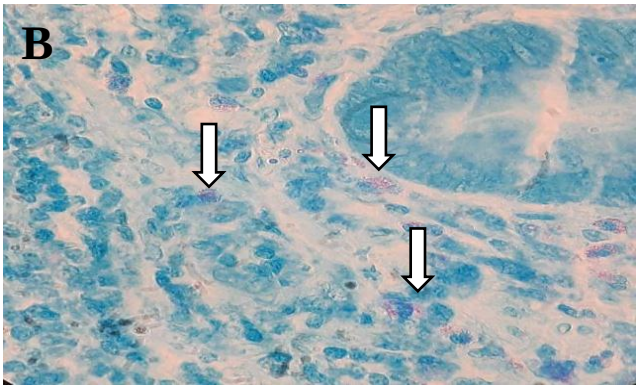




**Figure (9):** Appendicitis with increased number of eosinophils (arrows) infiltrating the lamina propria layer. (A) Cross section 400X, (B) Cross section 1000X (MGG) stains.

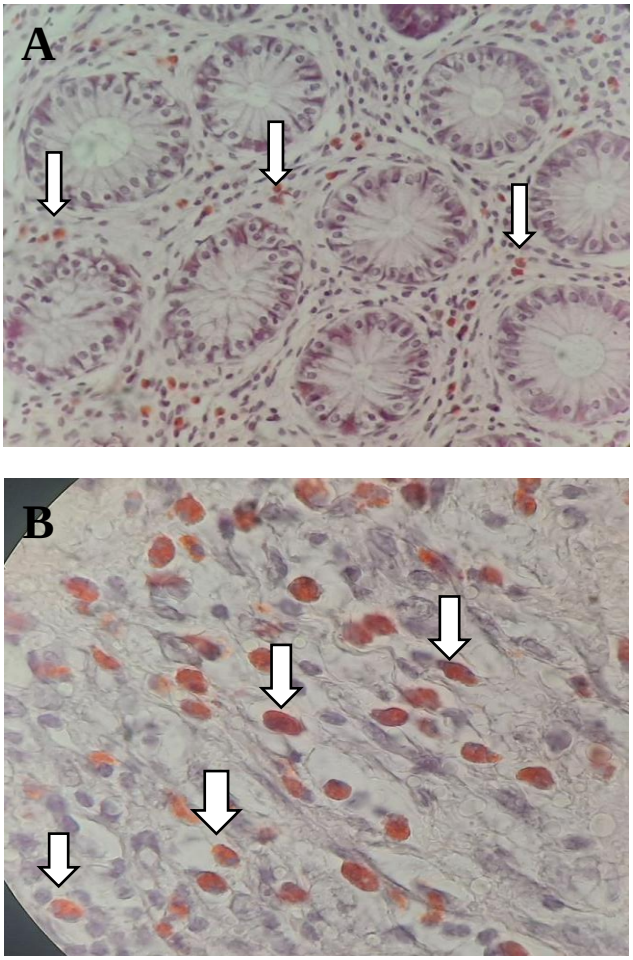



**Figure (10):** Appendicitis with increased number of eosinophils (arrows) infiltrating the lamina propria layer. (A) Cross section 400X, (B) Cross section 1000X (Congo red) stains.

## 5. CONCLUSIONS

We conclude that demonstration of eosinophil by Congo red more effective than MGG and H&E and there was a positive correlation between tissue and peripheral blood eosinophil. In acute appendicitis males were affected more than females and the high incidence of acute appendicitis was seen in age group (≤20 years).

Weight of appendices gradually increased with age but the dimensions decreased. The actual means of CBC (WBC, RDW and platelet) parameters were significantly different from theoretical means and there was non-significant sex difference in CBC parameters.

## Conflict of Interest (1)

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