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RESEARCH PAPER

Hormonal aspects of skin hyperpigmentation in healthy Kurdish primigravida women in Erbil City

Renas Najat Saleem and Sarbaz Ibrahim Mohammed

Department of Biology, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq

A B S T R A C T:

The present study aimed to determine some hormonal and biochemical aspects of skin pigmentation in pregnant women. The longitudinal study included forty-three healthy volunteers; ages ranged between 16-28 years, monitored from the beginning of gestation to delivery of birth. Then, women were divided into two groups (25 pigmented and 18 non-pigmented women) to evaluate hormonal and biochemical criteria. The result revealed a significant elevation in adrenocorticotropic hormones (ACTH), estrogen, progesterone hormone levels in pigmented women, and the progressing gestational period significantly changed the hormonal level, liver function parameters**.** At the same time**,** Alanine transaminase (ALT) reduced considerably in the first trimester. We concluded that skin pigmentation in primigravida women is associated with elevated ACTH, female sex hormones level, and liver enzymes. Hyperpigmentation was slightly evident in pregnant mothers with female fetuses than male fetuses.

KEY WORDS: ACTH; Primigravida; skin pigmentation; sex-hormones
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1.INTRODUCTION :

The most generally recorded skin change during gravidity is hyperpigmentation (refers to dark spots or patches on the skin), which upgrade in around 90% of pregnant ladies, especially during the second trimester of pregnancy. Different types of pigmentation such as melasma, chloasma, stretch marks, linea nigra, acne, spider angiomas and varicose veins appear on the different part of the body. Hormonal changes may induce pigmentation during gestation, enhancing a temporary elevation in the quantity of melanin secretion by melanocytes (Ibrahim *et al.*, 2020; Motosko *et al.*, 2017; Bieber *et al.*, 2017; Fernandes and Amaral, 2015).

Gravidity is identifying by a change in endocrine hormones, metabolic, vascular, and immunity systems, led to deferent physiological and pathological changes in skin, including changes in coloration, alterations of the connective tissue, vascular system, and hormonal function. Pigmentation is highly related with female sex hormones and medications with estrogens and progesterone, such as the contraceptive tablet or hormone substitution therapy (Friedman *et al.*, 2019; Hurst and Jennifer 2016; Tyler 2015; Muller and Rees 2014).

The melanin synthesis and distribution process is called melanogenesis. It synthesised in melanocytes present among the epidermis' basal cells. Pigments formed in melanocyte melanosomes are then stored in the basal layer of epidermal cells and dermal macrophages, which

* **Corresponding Author:**
Sarbaz Ibrahim Mohammed
E-mail: Sarbaz.mohammed@su.edu.krd
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become melanophores (Ibrahim *et al.,* 2020; Maranduca *et al.,* 2019; Chaudhary *et al.,* 2015).

The melanin is a color which is delivered by melanocytes at the degree of melanosomes (the melanogenesis). Both melanocyte stimulating hormones (MSH) and ACTH are produced by the cleavage of proopiomelanocortin (POMC) after corticotropin-delivering factor (CRF) incitement and afterward MSH follows up on the skin causing hyperpigmentation. Expanded movement of the maternal adrenal and pituitary organs and a commitment from the creating fetal endocrine organs, expanded cortisone levels, sped up metabolism, and upgraded creation of progesterone and estrogenic chemicals are liable for most skin changes in gestation (Friedman *et al.,* 2019; Rofiq *et al.,* 2019).

Pregnancy-specific changes are the leading cause of abnormal liver function test during the pregnant state, particularly in the third trimester (Mutua *et al.,* 2019; Mishra *et al.,* 2016). Pregnant ladies are especially powerless against iron reduction because of significant increment of iron demands during pregnancy to help the development of erythrocyte mass and plasma volume, and fetal-placental development (Loy *et al.,* 2019; Fisher and Nemeth 2017; Breymann, 2015).

The present study aims to investigate hormonal and biochemical changes that may cause different type pigmented area during pregnancy.

2. MATERIALS AND METHODS

2.1. Subjects

The longitudinal study included forty-three healthy Kurdish primigravida volunteer women examined. They were admitted to a maternity teaching hospital in Erbil, who attended routine care, monitored from the beginning of gestation to delivery of birth. The pregnant women aged between (16-28) years. Other information was taken from the pregnant women by questioners before starting to evaluate hormonal and biochemical analysis. Women divided into two groups; group A included 25 women with gestational pigmentation, and group B had 18 women with no gestational pigmentation.

2.2. Collection of blood samples

The blood samples were collected three times through experiment from every 43 pregnant women during each trimester of pregnancy at 1-13, 14-26, and 27-40 weeks, respectively. After obtaining the information, about seven (7) ml of venous blood was obtained from each woman every three months by vein puncture, using a ten (10 ml) disposable syringe; the blood was added into sterile, plain tubes the serum by centrifugation at 3000 rpm for 15 min. (at $4c^{o}$, using cooling centrifuge) to separate serum and divide it into different parts and was stored frozen at -80c̊ (Sony, Ultra-low, Japan) to estimate later biochemical and hormonal tests.

2.3. Hormonal Analysis

2.3.1 Determination of adrenocorticotropic hormone (ACTH)

The ACTH Ref 7023 Biomerica ELISA kit provides materials for the quantitative measurement of ACTH in plasma.

2.3.2. Determination of estradiol (E2), progesterone hormone, and β-human chorionic gonadotropin

The Accubind ELISA Kit (code: 4925-300), (code: 4825-300), and (code: 825-300) were used for the quantitative Determination of estradiol E2, progesterone, and β-human chorionic gonadotropin levels respectively in human serum.

2.4 Biochemical analysis

Biochemical tests such as ALT, AST, triglyceride, alkaline phosphate (ALP), serum albumin, total protein, total bilirubin and direct bilirubin kit were measured by using Cobas c111 analyzer-Roche Diagnosis.

2.5. Statistical analysis

All data expressed as Means ± Standard Error. Statistical analysis of the obtained data has done according to independent samples t-test, complete randomized design, and qi-square to compare the means of pigmentation and non-pigmentation groups in the study. The statistical analysis carries out using statistically available software (SPSS version 17), using Matlab program for preparing standard hormonal curves and significant level at ($P \leq 0.05$).

3- RESULTS

This cross-sectional study had done on the healthy Kurdish primigravida volunteer women attending the maternity teaching hospital in Erbil. A total of 43 (25 pigmented and 18 no pigmented) pregnant women at different gestational periods had registered in the study after taking informed consent. The hormonal and biochemical result shown in table (1, 2, 3, 4, and 5).

3.1. Hormonal analysis

The ACTH, estrogen, progesterone and hCG values had measured in pigmented and non-pigmented mothers and in the first, second, and

third trimester of gestation, as shown in tables 1& 2.

The results displayed a significant increase of plasma ACTH in the first, second, and third trimesters, while estrogen and progesterone increased significantly in the second and third trimester with non-significant increase in first trimester for progesterone only in pigmented skin when compared with non-pigmented mothers.

In the present studies, there was a significant increase in ACTH, estrogen, progesterone and hCG level in the second and third trimester when contrasted with the first trimester.

Table 1. Some hormonal variations between pigmentation and non-pigmentation in all trimesters of healthy Kurdish primigravida women.

Parameters	First Trimester		P-Value	Second Trimester		P-Value	Third Trimester		P-Value
	No Pigmentation	Pigmentation		No Pigmentation	Pigmentation		No Pigmentation	Pigmentation	
ACTH (pg/ml)	9.685 ± 1.588	14.37 ± 1.054	0.01	13.42 ± 1.291	21.46 ± 3.254	0.05	14.71 ± 1.236	24.63 ± 4.283	0.05
Estradiol (pg/ml)	23.18 ± 4.470	22.42 ± 3.550	NS	25.17 ± 3.783	35.06 ± 3.008	0.05	32.67 ± 3.506	45.07 ± 3.964	0.05
Progesterone (ng/ml)	2957.0 ± 90.4	3408.0 ± 457.6	NS	6451.0 ± 606.1	7768.0 ± 303.8	0.05	7397.0 ± 643.3	8760 ± 266.6	0.05
hCG (mIU/ml)	678.0 ± 164.1	753.5 ±111.1	NS	821.6 ± 156.0	996.2 ± 121.2	NS	1153.0 ± 110.7	1226.0 ± 93.05	NS

NS: Non-Significant

Table 2. Some hormonal variations during the gestational period of healthy Kurdish primigravida women.

Parameters	First Trimester	Second Trimester	Third Trimester
ACTH (pg/ml)	12.43 ± 0.986^{a}	18.13 ± 2.092^{ab}	19.97 ± 2.712^{b}
Estradiol (pg/ml)	22.74 ± 2.734^{a}	31.42 ± 2.575^{b}	37.87 ± 3.589^{b}
Progesterone (ng/ml)	3222 ± 451.3^{a}	7257 ± 330.2^{b}	7886 ± 346.8^{b}
hCG (mIU/ml)	722.2 ± 92.58^{a}	924.0 ± 95.63^{a}	1196 ± 70.28^{b}

A similar line letters mean no significant differences; the various letters of a similar line mean significant differences.

3.2. Biochemical parameters

3.2.1. Liver function test

The results in table 3 show no significant differences in the mean values of total bilirubin, direct bilirubin, alkaline phosphatase, AST, albumins, and total protein values between pigmented and non-pigmented primigravida women in all trimesters. Except ALT level decreased significantly ($p<0.05$) in pigmented women compared with non-pigmented women in the first trimester.

The results of table (4) indicate that albumin and AST levels reduced in both the second and third trimester but**,** ALT and total protein was decreased only in the third trimester when compared with the first trimester. On the other hand, total bilirubin was increased significantly in both the second and third trimester. In contrast, direct bilirubin and alkaline phosphatase increased significantly only in the third trimester when compared with the first trimester.

Table 3. Liver functional test variations between non-pigmentation and pigmentation in all trimesters of healthy Kurdish primigravida women

Parameters	First Trimester		P-Value	Second Trimester		P-Value	Third Trimester		P-Value
	No Pigmentation	Pigmentation		No Pigmentation	Pigmentation		No Pigmentation	Pigmentation	
Total Bilirubin (mg/dl)	0.370 ± 0.028	0.374 ± 0.028	NS	0.588 ± 0.051	0.6148 ± 0.057	NS	0.465 ± 0.031	0.491 ± 0.018	NS
Direct Bilirubin (mg/dl)	0.127 ± 0.032	0.154 ± 0.021	NS	0.251 ± 0.009	0.291 ± 0.026	NS	0.455 ± 0.179	0.434 ± 0.141	NS
AST (U/L)	23.38 ± 1.888	22.62 ± 1.527	NS	17.66 ± 1.360	19.04 ± 1.066	NS	16.22 ± 1.345	18.12 ± 0.776	NS
ALT (U/L)	18.73 ± 1.660	14.74 ± 0.983	0.5	15.77 ± 2.109	15.40 ± 1.437	NS	11.62 ± 1.717	11.68 ± 0.782	NS
Alkaline phosphatase	81.69 ± 4.741	82.98 ± 3.962	NS	112.6 ± 10.38	95.48 ± 6.162	NS	201.6 ± 20.98	220.2 ± 11.84	NS

(Ul/L)									
Albumine (g/dl)	4.362 ± 0.149	4.268 ± 0.062	NS	3.871 ± 0.089	3.975 ± 0.064	NS	4.111 ± 0.134	3.879 ± 0.076	NS
Total Protein (g/dl)	6.587 ± 0.237	6.633 ± 0.124	NS	6.436 ± 0.152	6.421 ± 0.168	NS	6.191 ± 0.261	5.780 ± 0.181	NS

NS: Non-Significant

Table 4. Liver functional test variations during gestational period of healthy Kurdish primigravida women

Parameters	**First Trimester**	**Second Trimester**	**Third Trimester**
Total Bilirubin (mg/dl)	0.372 ± 0.020^{a}	0.603 ± 0.039^{c}	0.480 ± 0.016^{b}
Direct Bilirubin (mg/dl)	0.143 ± 0.018^{a}	0.274 ± 0.016^{ab}	0.443 ± 0.109^{b}
AST (U/L)	22.94 ± 1.176^{b}	18.46 ± 0.838^{a}	17.32 ± 0.726^{a}
ALT (U/L)	16.41 ± 0.938^{b}	15.55 ± 1.200^{b}	11.65 ± 0.838^{a}
Alkaline phosphatase (Ul/L)	82.44 ± 3.005^{a}	102.6 ± 5.708^{a}	212.4 ± 11.10^{b}
Albumin (g/dl)	4.307 ± 0.071^{b}	3.932 ± 0.052^{a}	3.976 ± 0.072^{a}
Total Protein (g/dl)	6.614 ± 0.121^{b}	6.427 ± 0.115^{b}	5.952 ± 0.153^{a}

A similar line letters mean no significant differences; the various letters of a similar line mean significant differences.

3.3 Baby gender and skin pigmentation

The present work Chi-square test revealed a non-significant relation between baby gender and skin pigmentation. The female gender percentage in non-pigmented women was (33.3 %) while in pigmented women was (58.3 %). On the other hand, the percentage of male gender in non-pigmented women was (66.7 %) and in pigmented women was (41.7%), i.e. male gender percentage is twice the female gender in non-pigmented women.

Table (5): Relation between baby gender and skin pigmentation during normal pregnancy.

Baby gender	Females	Males	P-Value
No Pigmentation	6 (33.3%)	12 (66.7%)	0.098
Pigmentation	14 (58.3)	10 (41.7)	

4.DISCUSSION

4.1Pigmentation during pregnancy

The commonly encountered physiological changes during pregnancy are pigmentary alterations, the most common colouration being melasma, linea nigra, palmar erythema (Ciechanowicz *et al.*, 2018; Urasaki, 2010).

Many factors have associated with the development of pigmentation, including hormonal influences of pregnancy, like elevated ACTH levels, estrogen and progesterone, ultraviolet radiation. Other possible factors are hepatic dysfunction, some haematological changes, nutritional deficiency, genetics, and racial (Cario 2018; Costin and Hearing, 2007; Damoa *et al*., 2006).

4.2Hormonal Analysis

4.2.1 ACTH

Results show a highly significant elevation in serum ACTH levels of first, second, and third trimesters in normal Kurdish primigravida pigmented ladies when compared with ACTH levels of non-pigmented healthy women, the present result in agreement with (Sandru *et al.*, 2019; Maranduca *et al.*, 2019; Sanson *et al*., 2003) they showed that ACTH levels increased throughout pregnancy reaching maximum levels during the third trimester. There is agreement that the proopiomelanocortin (POMC) peptides with huge melanogenic action are ACTH, α-MSH, and β-MSH. ACTH invigorates skin pigmentation, prevalently in sun-uncovered zones through a mechanism of binding ACTH to the epidermal melanocytic specific receptor (MC1-R), increased tyrosinase activity leads to melanin synthesis in which enhances skin pigmentation because of contribution large number and higher susceptibility of melanocytes receptors to hormonal stimulus in these areas and may be due to that the progressive well investigated stresses of primigravida to near the term and delivery, cause ACTH secretion from the anterior pituitary gland to suppress stresses pathway and lead to enhance pigmentation process (Maranduca *et al.*, 2019; Brenner and Hearing, 2008).

The observation of high ACTH concentration in pigmented pregnant women may be due to that CRH and vasopressin stimulate the POMC-derived peptide. The CRH is recognized as an inducible endocrine hormone from the placenta in the net-physiology of pregnancy, influencing ACTH release from the anterior pituitary lobe, raising the possible role in influencing skin pigmentation (Maranduca *et al.*, 2019; Petraglia *et al.*, 2010 and Brenner and Hearing, 2008).

On the other hand, a previous publication focused on a relationship between parity and increased stress. In normal pregnancies, there is evidence of oxidative stress related to the lipid changes observed in pregnancy (Brody *et al*., 2011).

The effects of the gestational period on the ACTH ratios indicate a significant increase of ACTH level in the third trimester compared with the first trimester. This result in agreement with previous studies (Sandru *et al.*, 2019; Karaca *et al*., 2010; Lindsay and Nieman, 2005), summarising that women of childbearing age tend to have larger glands, and upward convexity of the pituitary gland causes increased ACTH releasing, also include placental synthesis and release of biologically active CRH and ACTH. Furthermore, they found a positive correlation has observed between CRH and POMC; Proopiomelanocortin (POMC) is the polypeptide precursor of ACTH, which is physiologically present in blood originate from the anterior pituitary. Indeed, CRH levels increase exponentially during the late trimester,

lead to more releasing of POMC and subsequence production of ACTH (Maranduca *et al.,* 2019; Sandru *et al.,* 2019).

4.2.2 Sex hormones

Maternal estrogen results show a significant increase in the second and third trimester of gestation in pigmented mothers, this is in agreement with other findings (Friedman *et al*., 2019; Mahmood *et al*., 2011; Brenner and Hearing, 2008), and they provide the role of estrogen and progesterone in the causation of discolored patches and hyperpigmentation during pregnancy. It is due to the fact of the estrogens mechanism by which it progresses pigmentation. Probably, there are specific regional differences in the number of melanocytes in the skin (Cario, 2018).

Furthermore, the stimulating effects of estrogen on proliferation and tyrosinase role of epidermal melanocytes enhance melanin synthesis markedly. Where it accumulates within melanosomes, tend to develop chloasma observe in gravidity mother, often accompanied by elevated coloration in different areas of the body, including the linea alba, perineal skin and the areola, the majority of which generally blur following parturition (Rofiq *et al.,* 2019; Alves *et al*., 2005; Slominski *et al*., 2004).

Our data study for serum estrogen and progesterone demonstrate a significant increase in the second and third trimester when comparison with first trimester, agree with researches of (Cario 2018; Mahmood *et al*., 2011, Zen *et al*., 2010; Villaseca *et al*., 2005) indicating that in healthy pregnant women, serum estrogen level progressively increased throughout gestation, due to that additive production sites for estrogen rather than ovaries, include the corpus luteum, the placenta, and the fetal adrenal glands. Elliott *et al*., (2004) argue that estrogen may have evolved a pivotal role in establishing, maintaining, and culminating pregnancy. Also, estrogen influences muscle strength and increases uterine muscle contractility and during the gestational term progresses delivery (Cario 2018; Kristiansson and Wang, 2001)

The present study findings for progesterone are inconsistent with the results reported by (Rofiq *et al.,* 2019; Bolanca *et al.,* 2008), recording significant maternal serum elevations in the second and third trimester when compared with the first trimester. Progesterone's adaptive increases by placenta predominate later during pregnancy and the initial sources of corpus luteum, fetal and maternal adrenal glands. Propounding progesterone promotes uterine muscle relaxation for maintenance of pregnancy and fetal development, loosens up smooth muscle, which causes atony of the gastrointestinal and urinary system. Also, together with estrogens, stimulate the mammary glands' growth in the breasts in preparation for lactation. The estrogen stimulates the secretion of melanocyte-stimulating hormones higher than normal levels during gestation, in turn, leads to melanin pigment production, which is responsible for general gestation-induced pigmentary phenomena (Friedman *et al.*, 2019).

Physiologically increase in the level of hCG shown in pigmented mother compared with non-pigmented mothers' results supported by conclusions of (Petraglia, *et al.,* 2010 and Sanson *et al*., 2003) who showed that hCG, produced in the placenta implicated as important endocrine mediators in the physiology of pregnancy and parturition. Plasma hCG concentration correlated positively with gestational age and increased placental mass.

Moreover, a significant increase in hCG has shown in the third trimester when comparison with first trimester inconsistent by (Gallego *et al*., 2010; Tran, 2006), reporting that the gestational-related increase of maternal hCG serum levels reaches as the peak during the first and third trimester, during to the placental mass increasing in late pregnancy.

4.3 Biochemical parameters

4.3.1. Liver function test parameters

The results showed non-significant differences in the mean values of total bilirubin, direct bilirubin, ALP, AST, albumin and total protein value between the pigmented and non-pigmented primigravida women in all trimesters, whereas, ALT level was decreased significantly in pigmented women in the first trimester.

These results were dissimilar with findings documented (Mutua *et al.,* 2019; Mishra *et al.,* 2016; Jamjute *et al*., 2009), who showed that ALT and AST levels were slightly elevated, indicating liver diseases, spider naevi, pruritus and palmar erythema that may occur during normal pregnancy.

The current results indicate that albumin level decreased in the second and third trimesters and total proteins in last trimester compared with the first trimester. The present result agrees with studies of (Khatun *et al.,* 2020; Venugopal and Rajamma 2015; Gohel *et al.,* 2013; Suresh and Radfar, 2004), who showed a decline of serum albumin in the first ten weeks of pregnancy and slightly decreased toward term because there was a high plasma volume, the yields elevate in plasma volume proceeds from 50 cc at ten weeks pregnancy to 800 cc at 20 weeks. Also, excessive loss of urinary excretion may explain the fall in blood albumin level through late gestation (Khatun *et al*., 2020; Gohel, *et al.*, 2013; Jamjute *et al*., 2009).

On the other hand, the result of ALT, AST, total bilirubin, and direct bilirubin were dissimilar with findings documented (Khatun *et al.,* 2020; Venugopal and Rajamma 2015; Gohel, *et al.*, 2013) they showed serum ALT, AST concentration significantly high while serum total bilirubin, and direct bilirubin concentration as gestation advanced were due to a phenomenon called hemodilution could be responsible for the change in serum liver enzymes or liver damage.

Similar results found previously by (Mishra *et al.*, 2016; Venugopal and Rajamma 2015; Gohel, *et al.*, 2013; Tran 2006) reporting shifts in protein level and change gradually throughout gestation because the metabolic demands increased due to physiological and hormonal changes in the mother and growth of the fetus. Further explanation is that during gestation, micronutrients deficiencies may have severe consequences for pregnancy outcomes and, as a resulting decline, blood contents of proteins, vitamins and minerals (Khatun *et al.,* 2020; Venugopal and Rajamma 2015; Guerra *et al*., 2009).

The present study shows significantly higher alkaline phosphatase values in the third trimester and total bilirubin increased only in the second trimester compared with the first trimester. In agreement with (Mutua *et al.,* 2019; Mishra *et al.*, 2016; Suresh and Radfar, 2004), they summarised that alkaline phosphatase value ascendancy was at the binging of the last trimester. This increase is due to the leakage of placental alkaline phosphatase into the maternal blood (Mutua *et al.*, 2019; Mishra *et al.,* 2016; Suresh and Radfar, 2004)

Serum ALP concentration directly forwardly matches skeletal and fetus development and mineral storage in the maternal organs. Biological alterations in alkaline phosphatase value happen with regularity during the reproductive cycle. The greatness of alteration corresponds largely to anatomic growth and development (Khatun *et al*., 2020).

The result of total bilirubin and direct bilirubin in the current study agrees with previous investigations (Mishra *et al.,* 2016; Suresh and Radfar, 2004) show a significant elevation from the first to the third trimester. Hemodilution partially responsible for the diminishing of bilirubin levels because albumin is the protein carrier of bilirubin; decreased albumin concentrations during normal pregnancy interpret gestational bilirubin declines (Khatun *et al.,* 2020; Alonso, 2006)

4.4 Baby gender and skin pigmentation

The chi-square test has shown a non-significant correlation between baby gender and skin pigmentation in the present study. Ibrahim *et al.*, (2020) confirmed these results showed that chloasma is more likely to occur among female fetus than males, while Abdullah (2020) showed that hyperpigmentation was more evident in primigravida pregnant mothers with male fetuses than female fetuses. It may be related to hormonal, or any other haematological variation between these pregnancy cases and this must be confirmed and further studied.

CONCLUSIONS

The present research concluded the presence of ACTH and female sex hormones great effects in pregnancy women hyperpigmentation. Estimation in serum liver enzyme in all gestation trimesters is important for the diagnosis liver disease and anemia during gestation. Hyperpigmentation was more evident in pregnant mothers with female fetuses than male fetuses.

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