ORIGINAL ARTICLE

THE INFLUENCE OF SICKLE CELL ANEMIA ON LH, FSH, AMH, ESTRADIOL, VITAMIN D AND FERRITIN LEVELS OF SUDANESE FEMALES

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ABSTRACT

Background: As medical advances improve survival, reduce disease-related morbidity and improve quality of life; reproductive issues and fertility will take higher priority in sickle cell disease community, because every hundreds of thousands around world die from diseases caused by anemia. **Objectives:** This is a case control study was done to evaluate Estradiol, FSH, LH, serum Ferritin, AMH and Vitamin D levels among Sudanese females with sickle cell anemia, sickle cell trait and their correlations with duration of hydroxyurea treatment, blood transfusion and age. **Methods:** ELISA and Mindary MR-96A auto analyzer were used for evaluation. **Results:** Results were done by SPSS, showed that Sudanese females with sickle cell disease in comparison with females without sickle cell disease there were significantly increased in means concentration of serum (FSH, Ferritin) with P-value =(0.006, 0.000) respectively, significantly decreased in mean concentration of (Vitamin D) with P-value = (0.020), and insignificant differences in means concentration of serum (AMH, LH and Estradiol) with P-value = (0.862, 0.947, 0.440) respectively. Also the subjects of diseased group were divided into two sub groups, sickle cell disease and sickle cell trait groups according to the electrophoresis bands, the results found that there were significant increase in means concentration of (FSH and Ferritin) with P-value = (0.044, 0.032) and (0.000, 0.005) respectively, and there were insignificant differences in means concentration of (AMH, LH and Estradiol) with P-value = (0.997, 0.963), (0.916, 0.865) and (0.9, 0.8) respectively between sickle cell disease, sickle cell trait and control groups. In addition the results showed that there was insignificant difference in mean concentration of (Vitamin D) of females with sickle cell Trait in comparison with females without sickle cell disease P-value = (0.978). **Conclusions:** The concentration of serum FSH, Ferritin increased, concentration of Vitamin D decreased and there were insignificant difference between mean concentrations of AMH, LH & Estradiol among Sudanese females with sickle cell disease and sickle cell trait in comparison to healthy individuals. There was insignificant difference in mean concentration of Vitamin D of females with sickle cell Trait in comparison to control. There were negative correlation between the FSH and LH concentration and duration of hydroxyurea treatment, and there were no correlation between the Vitamin D, AMH, Ferritin and Estradiol concentration and duration of hydroxyurea treatment among Sudanese females patients. There were positive correlation between FSH and LH concentration and age, negative correlation between Vitamin D level and age and no correlation between AMH, Estradiol and Ferritin concentration and age among patients. There were no correlation between FSH, LH, Estradiol and Ferritin concentration and blood transfusion among Sudanese females with sickle cell anemia.

Keywords: *Reproductive issue, Fertility, Hydroxyurea.*

1.INTRODUCTION

Sickle cell disease (SCD) is an inherited autosomal recessive hemoglobinopathy characterized by chronic hemolytic anemia; (Firth & Head, 2004) suggested that genetic disorder caused by a point mutation in beta globin gene which results in the substitution of valine for glutamine [1]. Isaacs and Hayhoe (1967) suggested that the resultant hemoglobin variant HbS polymerizes at low oxygen tension causing the characteristic sickle deformity of the red cell, the main aetiopathogenetic feature of this disease [2]. This may block different areas of the microcirculation or large vessels, causing infarcts of various organs and tissue, ischemia, chronic organ damage and organs dysfunction including endocrine organs, erectile dysfunction, osteoporosis, thyroid dysfunction and gonadal failure, as Whitley (2014) suggested that abnormal shape of red blood cells make it difficult for it to flow normally through small blood vessels, this process can affect reproductive organs and impact fertility, etiologies of impaired male fertility are multifactorial and include hypogonadism, sperm abnormalities and complication of medical therapies, but much less is known about the prevalence and etiology of infertility in women with sickle cell anemia [3].

So clinically it is very important to evaluate gonadotropins, gonadal hormone, ferritin and vitamin D among females with sickle cell anemia.

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are called gonadotropins, as Tamara et al. (2009) suggested that they stimulate the gonads - in males, the testes, and in females, the ovaries [4].

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Follicle-stimulating hormone (FSH): is a gonadotropin, a glycoprotein poly peptide hormone. Parsons (2014) suggested that FSH is synthesized and secreted by the gonadotropin cells of the anterior pituitary gland, and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system [5]. Fowler et al. (2003) suggested that in females FSH initiates follicular growth, specifically affecting granulosa cells. With the concomitant rise in inhibit B, FSH levels then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the luteal phase, there is a slight rise in FSH that seems to be of importance to start the next ovulatory cycle [6]. High levels of Follicle-Stimulating Hormone indicate that the normal restricting feedback from the gonad is absent, leading to an unrestricted pituitary FSH production.

Luteinizing hormone (LH): also known as (lutropin and sometimes lutrophi) is a hormone produced by gonadotropic cells in the anterior pituitary gland. Makoto et al. (1992) suggested that in females, an acute rise of LH ("LH surge") triggers ovulation and development of the corpus luteum [7]. Effects in females: LH supports theca cells in the ovaries that provide androgens and hormonal precursors for estradiol production. LH is necessary to maintain luteal function for the second two weeks of the menstrual cycle. If pregnancy occurs, LH levels will decrease.
FSH and LH levels are normally low during childhood and, in females, high after menopause.
At molecular level as Bèdècarrats et al. (2003) suggested that the gonadotropin gene expression is regulated by Mullerian inhibiting substances MIS [8].

Anti-mullerian hormone (AMH) or Mullerian inhibiting substance (MIS): Cate et al. (1986) suggested that AMH is a protein hormone structurally related to inhibin and activin, and a member of the transforming growth factor-β (TGF-β) superfamily of growth and differentiation factors, it is a dimeric glycoprotein, that in humans is encoded by the AMH gene [9]. Behringer (1994) suggested that it inhibits the development of the mullerian ducts "paramesonephric ducts" in male embryo [10]. Münsterberg and Badge (1991) suggested that İn the absence of AMH, the Müllerian ducts develop into the uterus, fallopian tubes and the upper part of the vagina [11], so AMH expression is critical to sex differentiation at a specific time during fetal development. Rooij et al. (2002) suggested that AMH expression also occurs in ovarian granulosa cells of females postpartum and serves as an excellent molecular biomarker for relative size of the ovarian reserve and ovarian aging [12]. Visser et al. (2006) suggested that AMH is considered as a newer marker for ovarian function [13]. In addition Cimino and Casoni (2016) suggested that AMH plays crucial roles in sexual differentiation and gonadal function [14], because Bèdècarrats et al. (2003) suggested that AMH regulate gonadotropin gene expression [15], and production of sex hormones.
Grynberg et al. (2012) suggested that AMH expression can be differentially regulated by Estradiol depending on Estrogen receptors ES [16].

Estradiol: is a female sex hormone that is the predominant estrogen throughout female's reproductive years; this hormone has a significant impact or reproductive and sexual function as well as on other organs.
A key which is needed by the body to create sex hormones is Vitamin D. And recently Irani and Merhi (2014) suggested that in human granulosa cells, the altering AMH signaling, FSH sensitivity and progesterone production and release, all are controlled by Vitamin D, indicating a possible physiologic role for vitamin D in ovarian follicular development and luteinization [17].

Vitamin D: Kawchak et al. (2007) suggested that vitamin D refers to group of fat soluble sec steroids responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc [18]. Gupta et al. (2011), Glover et al. (2012) suggested that Vitamin D is required for bone minerilization, plays an important role in several physiologic system and act as anti-c ancer agent, vitamin D deficiency may be a risk factor for increased knee osteoarthritis pain [19, 20]. Holick (2012) suggested that Vitamin D deficiency is associated with increased respiratory infection, muscle weakness and increased risk of Falls and micro lesion [21], Serarslan et al. (2010) suggested that in children with sickle cell anemia whose bone are affected by infection osteoporosis and osteonecrosis vitamin D deficiency may be worsen bone condition [22]. Anne et al. (2004) suggested that Vitamin D is now recognized as one of the most common nutritional conditions among persons with SCD [23]. Hollis and Wagner (2004) suggested that Vitamin D is so essential to fertility because it is needed to help the body create sex hormones, some study found that nearly 40% of the women who had ovulatory dysfunction also had a clinical deficiency in vitamin D, and therefore it is easy to understand how important vitamin D is to a woman's fertility [24].
Finally to prevent hypogonadism which developed from iron overload we have to assess the level of serum Ferritin in females with sickle cell anemia.

Ferritin: Ferritin is a universal intracellular protein that stores iron and releases it in a controlled fashion the protein is produced by almost all living organism. Anna et al. (2004) suggested that ferritin acts as a buffer against iron deficiency and iron overload [25]. Ferritin is found in most tissues as a cytosolic protein, but small amount are secreted into the serum where it function as an iron carrier. Wang et al. (2010) suggested that Plasma Ferritin is also an indirect marker of total amount of iron stored in the body; hence serum Ferritin is used as a diagnostic test for iron deficiency anemia [26].

2. MATERIALS AND METHODS

2.1 Study design: This is a descriptive analytical case control study.

2.2 sample size and sampling technique: About 2ml of venous blood was collected from each patient in plain containers, after clotting, centrifuged for 3 minutes at 3000 RPM to obtain serum, then stored at (-20 to – 80) °C and then analyzed.

2.3 reagents: 8 levels of Calibrators, Calibrator Diluent, Microtiter plates, Wash solutions, Reagents A & B, Diluents A & B, Substrate solutions, Stop solutions & Product inserts.
Antibody coated microtiter plate with 96 wells, Enzyme conjugate Reagent, Ferritin reference standards, TMP Reagent and Stop Solution.

2.4 ethical considerations: Individuals who voluntarily accepted to participate in the study were included.

2.5 quality control: Controls were assayed at levels in the Low, Normal and High QC range. Quality control charts were maintained to follow the performance of the supplied reagents. Pertinent statistical methods were employed to ascertain trends. Significant deviation from established performance could indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents were used to determine the reason for the variations.

2.6. principle 1: The Fortress (LH, FSH, AMH, Estradiol and Vitamin D) Elisa assays were used a monoclonal antibody and (LH, FSH, AMH, Estradiol and Vitamin D) HRP conjugate in anti (LH, FSH, AMH, Estradiol and Vitamin D) coated plates. After incubation and subsequent washing the wells were incubated with a substrate solution. Measurements were performed after stopping the reaction used an acidic stop solution.
- ELISA reader Mindary MR-96A auto analyzer was used.
- Fortress diagnostics laboratory materials were used. ISO 13485 accredited company.

2.7 principle 2: Human Ferritin ELISA Kit Protocol was used. The Ferritin Quantitative Test based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilized one rabbit anti-ferritin antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horse-radish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the ferritin molecules being sandwiched between the solid phase and enzyme linked antibodies. After a 45-minute incubation at room temperature, the wells were washed with water to remove unbound-labeled antibodies. A solution of TMP Reagent was added and incubated at room temperature for 20 minutes, resulted in the development of a blue color. The color development was stopped with the addition of Stop solution and the color was changed to yellow and measured spectrophotometrically at 450 nm. The concentration of ferritin was directly proportional to the color intensity of the test sample.
- ELISA reader was used.
- The Phonex Pharmaceuticals, INC. Human Ferritin ELISA Kit Protocol was used

2.8 study site: The study was conducted in sickle cell anemic Sudanese females in Jaafer Ebn Ouf hospital in Khartoum state and Hejleej hospital in western Kordofan state.

3.9 patients: This study included 50 Sudanese sickle cell anemic females and 30 healthy individuals as control (age was matched in the groups with range 2-38 years).

. Inclusion criteria: Sudanese females with sickle cell anemia were included.
. Exclusion criteria: Icteric or hemolyzed samples as well as Individuals with hyperlipidemia, hypertension, hyper or hypothyroidism, renal disease, bone diseases or any other disorders that may affect the levels of LH, FSH, AMH, Estradiol, Vitamin D and Ferritin Levels were excluded.

2.10 statistics: Analysis of the data was done by using SPSS computer program.

3. RESULTS

Fifty Sudanese females with sickle cell anemia were enrolled in this study to assess the influence of sickle cell disease on gonadotropins hormones (FSH & LH); gonadal hormones (Estradiol & AMH), Vitamin D levels in addition to Ferritin. And thirty Sudanese females without sickle cell anemia were served as control group. The statistical analysis was done by using SPSS computer program and the results were presented in as follow:

Table 1: The table shows the comparison between means concentration of Estradiol, Ferritin, FSH, LH, AMH and Vitamin D levels in Sudanese females with sickle cell disease and non-sickle cell disease.

Variables (ng\ml)	Mean ± SD in sickle cell females	Mean ± SD in non-sickle cell females	p-value
Estradiol	59.42±41.24	70.78±89.00	0.440
Ferritin	348.9±243.6	62.9±79.4	0.000
FSH	5.82±4.26	3.80±2.09	0.006
LH	4.61±3.23	4.52±3.43	0.947
AMH	0.697±0.449	0.684±0.226	0.862
Vitamin D	27.07±15.95	35.18±12.09	0.020

FSH : follicle-stimulating hormone; **LH**: Luteinizing hormone; **AMH:** Anti-Mullerian Hormone.

Independent sample T-test was used for comparison, value consider significant at level ≤ 0.05. There were significantly increase in means concentration of serum (FSH, Ferritin), significantly decrease in mean concentration of (Vitamin D) and there were insignificant difference between means concentration of (AMH, LH & Estradiol) among Sudanese females with sickle cell disease and healthy individuals.

Table 2: Comparison between means of AMH, LH, FSH, Estradiol and Ferritin levels in Sudanese females with sickle cell disease, sickle cell trait and non-sickle cell disease (Control).

Variables (ng\ml)	Groups	Mean ± SD	*p-value*
AMH	Sickle cell diseased	0.691±0.499	0.997
	Trait	0.720±0.192	
	Control	0.684±0.226	0.963
LH	Sickle cell diseased	4.244±4.189	0.916
	Trait	3.046±1.865	
	Control	3.833±2.628	0.865
FSH	Sickle cell diseased	5.541±2.394	0.044
	Trait	6.871±3.751	
	Control	3.800±2.093	0.032
Estradiol	Sickle cell diseased	58.4±44.9	0.9
	Trait	63±22	
	Control	59.2±57.7	0.8
Ferritin	Sickle cell diseased	368±249	0.000
	Trait	260±224	
	Control	46.8±39	0.005

LH: Luteinizing hormone; **FSH**: follicle-stimulating hormone; **AMH:** Anti-Mullerian Hormone.

One-Way ANOVA test was used for comparison, value is considered significant at level ≤ 0.05. There were significantly increase in means concentration of (FSH and Ferritin) and there were insignificant differences in means concentration of (AMH, LH and Estradiol among Sudanese females with sickle cell anemia and sickle cell trait in comparison to healthy individuals.

Table 3: Table shows comparison between means of Vitamin D level in Sudanese females with sickle cell trait and non-sickle cell disease.

Variable		Mean ± SD	P-value
Vitamin D (ng/ml)	Trait	21.84 ±13.58	0.978
	Control	21.96 8.58	

Independent sample T-Test, p-value ≤ 0.05 considered significant. There was insignificant difference in means concentration of (Vitamin D) of females with sickle cell Trait in comparison with females without sickle cell disease.

Table 4: Correlation between FSH, LH, Estradiol, Ferritin, AMH, Vitamin D levels and duration of hydroxyurea treatment among Sudanese females with sickle cell anemia.

	FSH (ng/ml)	LH (ng/ml)	Estradiol (ng/ml)	Ferritin (ng/ml)	AMH (ng/ml)	Vitamin D (ng/ml)
R	-o.447	-0.538	0.32	0.05	0.185	-0.107
P-value	0.04	0.039	0.20	0.84	0.510	0.694
Degree of Significant	Correlation Significant	Correlation Significant	Moderate correlation NS	No correlation NS	No correlation NS	No correlation NS

FSH: follicle-stimulating hormone; **LH**: Luteinizing hormone; **AMH:** Anti-Mullerian Hormone; **NS:** No Significant.

There were negative correlation between the (FSH and LH) concentration and duration of hydroxyurea treatment and there were no correlation between the (Vitamin D, AMH, Ferritin and Estradiol) concentration and duration of hydroxyurea treatment among Sudanese females with sickle cell anemia.

Table 5: The table presents the correlation between Estradiol, Ferritin, FSH, LH, AMH, Vitamin D levels (ng/ml) and age among Sudanese females with sickle cell anemia.

	Estradiol	Ferritin	FSH	LH	AMH	Vitamin D
R	0.203	0.11	0.316	0.405	-0.001	-0.445
P-value	0.166	0.42	0.047	0.009	0.993	0.005
	No correlation	No correlation	Correlation	Correlation	No correlation	Correlation
Degree of Significant	NS	NS	S	S	NS	S

FSH: follicle-stimulating hormone; **LH**: Luteinizing hormone; **AMH:** Anti-Mullerian Hormone.

There were positive correlation between (FSH and LH) concentration and age, negative correlation between (Vitamin D) level and age and there were no correlation between (AMH, Estradiol and Ferritin) concentration and age among Sudanese females with sickle cell anemia.

Table 6: The table presents the correlation between FSH, LH, Estradiol, Ferritin concentration and times of blood transfusion among Sudanese females with sickle cell anemia.

	FSH (ng/ml)	LH (ng/ml)	Estradiol (ng/ml)	Ferritin (ng/ml)
R	-.079	0.14	0.066	-0.027
P-value	0,7	0.5	0.8	0.9
Interpretation	No Correlation	No Correlation	No Correlation	No Correlation
Degree of Significant	NS	NS	NS	NS

FSH: follicle-stimulating hormone; **LH**: Luteinizing hormone; **NS:** No Significant.

There were no correlation between (FSH, LH, Estradiol and Ferritin) concentration and blood transfusion among Sudanese female with sickle cell anemia.

4. DISCUSSION

Sickle cell anemia leads to multi-organ dysfunction such as gonadal failure, which may affect reproductive organs and impact fertility.

The results showed that there were significantly increase in means concentration of serum (FSH, Ferritin) of females with sickle cell disease in comparison with females without sickle cell disease, with p-value of (0.006, 0.000) respectively, and significantly decrease in mean concentration of (Vitamin D) of females with sickle cell disease in comparison with females without sickle cell disease, with P-value of (0.020). And showed that there were insignificant differences in means concentration of serum (AMH, LH and Estradiol) of females with sickle cell disease in comparison with females without sickle cell disease, with p-value of (0.862, 0.947, 0.440) respectively. This result agreed with result done by Hagag et al. (2015) suggested that gonadal hormone in Egyptian female children with sickle cell anemia in correlation with iron overload with the p.value of (FSH, 0.021; Ferritin, 0.001) [27].

This result disagreed with result done in Saudi Arabia by Al-hazmi et al. (1992) suggested that endocrine disorder in patients with sickle cell anemia in female human volunteers showed that the FSH and LH had significantly lower in females with sickle cell anemia than non-sickle cell anemia. And Patients with the severe form of the sickle cell disease showed more frequent abnormalities of LH, FSH, cortisol and testosterone in comparison with the patients with a mild disease [2]. Other study also disagreed with this result done by Nashwa et al. (2009) suggested that female patients with SCD (Group 2) have a significant lower level of LH than the control group (8.72±5.44, 16.2±2.74 (ng/ml)) respectively p.value = (0.001). There was no significant difference between the level of FSH among Group 2 and the control group (6.19±3.60, 6.4±1.3 (ng/ml)) respectively p.value = (0.05) [29]. This different from another study result from different of population the population in this study takes the omega3 as treatment for long time it has strong effect on endocrine.

This result disagreed with result done by Hagag et al. (2015) suggested that gonadal hormone in Egyptian female's children with sickle cell anemia in correlation with iron overload, with the p-value of LH (0.003) [30]. This result agreed with Buison et al. (2004) suggested that the frequency of low serum levels of vitamin D in the group with sickle cell anemia exceeded the frequency found in the healthy group [31]. This result also disagreed with result done by Elchuries et al. (2015) suggested that the effects of hydroxyurea and bone marrow transplant on AMH level in females with sickle cell anemia; showed that AMH levels in sickle cell anemic patients who treated by hydroxyurea was <5th percentile for age-matched controls which defined as diminished ovarian reserve (DOR) [32].

The contradict finding might be justified by doses of new treatment called omega3 was taken continuously with folic acid in our study, as Nehra et al. (2012) suggested that omega3 has an effective role in prolonging the female reproductive lifespan and improving egg quality [33]. So in our study there was no significant difference of (serum AMH, LH and Estradiol) in sickle cell disease females compared to control. Rooij et al. (2002) suggested that AMH is a marker for ovarian reserve, ovarian aging [34]. And Garcia et al. (2009) suggested that AMH is a marker for oocyte quality [35]. Burger et al. (2008) suggested that Estradiol has the key role of ovarian function [36]. Filicori (1999) suggested that LH has a role in folliculogenesis and oocyte maturation [37].

Also in this study, the subjects of diseased group were divided into two sub groups, sickle cell disease and sickle cell trait groups according to the electrophoresis bands, the results found that there were significantly increase in means concentration of (FSH and Ferritin) between sickle cell disease, sickle cell trait and control groups with *p*-value of (0.044, 0.032) and (0.000, 0.005) respectively, which indicates that the sickle cell disease effects on the FSH and Ferritin levels. And there were insignificant differences in means concentration of (AMH, LH and Estradiol) between sickle cell disease, sickle cell trait and control groups with *p*-value of (0.997, 0.963), (0.916, 0.865) and (0.9, 0.8) respectively, which indicates that the sickle cell disease doesn't affect the AMH, LH and Estradiol levels.

In addition the results showed that there was insignificant difference in mean concentration of (Vitamin D) of females with sickle cell Trait in comparison with females without sickle cell disease, with p-value of (0.978). Person's correlation results showed that, there were negative correlation between the (FSH and LH) concentration and duration of hydroxyurea treatment among Sudanese females with sickle cell anemia with *p*-value of (0.04, 0.039) respectively. And there were no correlation between the (Vitamin D, AMH, Ferritin and Estradiol) concentration and duration of hydroxyurea treatment among Sudanese females with sickle cell anemia with *P*-value of (0.694, 0.510, 0.84, 0.20) respectively.

Person's correlation results showed that, there was negative correlation between (Vitamin D) level and age among Sudanese females with sickle cell anemia with P-value of (0.005), there were positive correlation between (FSH and LH) concentration and age among Sudanese females with sickle cell anemia with P-value of (0.047, 0.009) respectively. And there were no correlation between (AMH, Estradiol and Ferritin) concentration and age among Sudanese females with sickle cell anemia with P-value of (0.993, 0.166, 0.42) respectively. Person's correlation results showed that, there were no correlation between (FSH, LH, Estradiol and Ferritin) concentration and blood transfusion among Sudanese females with sickle cell anemia with P-value of (0.7, 0.5, 0.8, 0.9) respectively.

5. CONCLUSION

The study results finding revealed that:

- There were significantly increased in means concentration of serum (FSH, Ferritin), significantly decreased in mean concentration of (Vitamin D) and there were insignificant difference between means concentrations of (AMH, LH & Estradiol) among Sudanese females with sickle cell disease and healthy individuals.
- There were significantly increase in means concentration of (FSH and Ferritin) and there were insignificant differences in means concentration of (AMH, LH and Estradiol among Sudanese females with sickle cell anemia and sickle cell trait in comparison to healthy individuals.
- There was insignificant difference in mean concentration of (Vitamin D) of females with sickle cell Trait in comparison with females without sickle cell disease.
- There were negative correlation between the (FSH and LH) concentration and duration of hydroxyurea treatment, and there were no correlation between the (Vitamin D, AMH, Ferritin and Estradiol) concentration and duration of hydroxyurea treatment among Sudanese females with sickle cell anemia.
- There were positive correlation between (FSH and LH) concentration and age, negative correlation between (Vitamin D) level and age and there were no correlation between (AMH, Estradiol and Ferritin) concentration and age among Sudanese females with sickle cell anemia.
- There were no correlation between (FSH, LH, Estradiol and Ferritin) concentration and blood transfusion among Sudanese females with sickle cell anemia.

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