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Serum Level of Anti-mullerian Hormone among Sudanese Females with Sickle Cell Anemia in Khartoum and Western Kordofan States

مستوى الهورمون المضاد لمولر في مصل الدم لدي الإناث السودانيات المصابات بالأنيميا المنجلية في ولايتي الخرطوم وغرب كردفان

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Dedication

ToSoul of my wonderful deeply missed mom

Fayza

To Soul of my little angel son

Mumin

Ask my god to make your souls at highest paradise & better place

Feeling happiness

To all my family and any person who helped me to fulfill this research

I remember

How you helped me to grow with love, truth and honesty

How to choose the right path with values, morals and self-worth

How you gave me dreams with hope and confidence

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Abstract

Sickle cell anemia is widely spread throughout the world and the effects of it on human health are serious. It is a group of genetically passed down blood disorders. It results in an abnormality in the oxygen-carrying protein hemoglobin found in red blood cells.

This is a case control study was done inJaaferEbinOuf hospital in Khartoum state and Hejleej hospital in Western state during March to December 2016, to evaluate serum Anti-mullerian Hormone "AMH" level among Sudanese females with sickle cell anemia.

Eighty Sudanese females 50 females with sickle cell disease were selected as test group (40 females with sickle cell anemia & 10 females with sickle cell trait) and 30 healthy females without sickle cell disease as control group"age was matched. blood specimen were collected from both groups and serum anti-mullerian hormone (AMH) is determined by using Mindary MR-96A autoanalyzer.

The statistical analysis was done by using SPSS computer program, the results showed that there was insignificant difference in mean concentration of serum AMH of females with sickle cell disease(0.697±0.449)in comparison with females without sickle cell disease(0.684±0.226), with *p.value* of (0.862)

The result showed there was no correlation between the AMH concentration and age with (r=0.001,*p*-value =0.993). Also the results showed that there were no correlation between the AMH concentration and duration of hydroxyurea treatment with(r=0.185, *p*-value =0.510).

The study results revealed that that sickle cell anemia doesn't affect serum AMH level in females. Also there were no correlation between the AMH concentration, age and duration ofhydroxyurea treatment.

مستخلص الدراسة

مرض الخلية المنجلية او ما يعرف بمرض الأنيميا المنجلية,ينتشر حول العالم وله تأثيراته على صحة الانسان ويعتبر من أحد الأمراض الجينية التي تسبب خلل في الدم وتنتج عن إختلال في البروتين الناقل للاوكسجين (الهيموغلوبين) في خلايا الدم الحمراء.

أجريت هذه الدراسة للمقارنة بين مجموعتي المرضي والأصحاء في الفترة من مارس الي ديسمبر 2016 تم اختيارهم عشوائيا من مستشفي جعفر بن عوف بولاية الخرطوم ومستشفى هجليج بولاية غرب كردفان لتحديد مستوي هورمون AMH لدي مرضي الأنيميا المنجلية من فئة الإناث وتم قياسه باستخدام ELISA.

شملت هذه الدراسة 80 أنثى سودانية , منهن 50 مصابات بمرض الأنيميا المنجلية (40 منهن يعانون من مرض الأنيميا المنجلية و10 حالات حاملين للمرض (trait)) و 30 أصحاء ظاهريا كمجموعة تحكم . تم إجراء التحليل الإحصائي باستخدام برنامج SPSS وأظهرت النتائج عدم تأثر متوسط مستوي هورمون AMH لدي المرضي (0.449±0.697)عند مقارنتها بمجموعة التحكم (0.226±0.684) مع القيمة المعنوية (0.862) وأيضا أظهرت الدراسة أنه لا توجد اي علاقه ملحوظه بين مستوي هورمون AMH والعمر(r=0.001) والقيمة المعنوية (0.993), وفترة العلاج (r=0.185) والقيمة المعنوية (0.510).

خلصت الدراسة الي أن مرض الأنيميا المنجليه لا يؤثر تأثير ملحوظ في مستوي هورمون AMH في مصل الدم لدي الإناث, وأيضا لا توجد علاقة بين مستوي هورمون AMH والعمر وفترة العلاج.

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List of Abbreviations

AFC	Antral Follicle Count
AMH	Anti-Mullerian Hormone
APH	Anti Paramesonephric Hormone
DAX1	Dosage sensitive sex reversal (DSS), Adrenal hypoplasia congenital (AHC) critical region on the X chromosome gene 1 .
DNA	Deoxyribo-Nucleic acid
DOR	Diminished Ovarian Reserve
EC	European Committee
ELISA	Enzyme Linked Immune Sorbent Assay
FSH	Follicle Stimulating Hormone
G6PD	Glucose 6 Phosphate Dehydrogenase
GnRH	Gonadotropin releasing hormone
Hb A	Hemoglobin A
Hb F	Hemoglobin F
Hb S	Hemoglobin S
Hb SC	Hemoglobin SC
Hb SS	Hemoglobin SS
IVF	In Vitro Fertilization
MIF	Mullerian Inhibiting Factor
MIH	Mullerian Inhibiting Hormone
MIS	Mullerian Inhibiting Substance
PO2	Partial pressure of Oxygen
RBCs	Red Blood Cells
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease

SCT	Sickle Cell Trait
SD	Standard deviation
SF1	Steroidogenic Factor 1
SOX9	Sry-type HMG box genes 9
TGF-β	Transforming Growth Factor-β

Chapter one

Introduction and literature review

1. Introduction and Literature review

1.1. Introduction

Sickle cell anemia is one of the genetic disorders of hemoglobin, it is inherited disease caused by reduced or abnormal synthesis of globin.In sickle cell anemia a point mutation resulting from a single nitrogenous base change in the DNA coding for the amino acid in the sixth position in the beta globin chain (Adenine is replaced by Thymine), this leads to an amino acid change from Glutamic acid to Valine, which results in abnormal hemoglobin called hemoglobin S (Hb S) sickle cell hemoglobin (Firth & Head, 2004).

The presence of Hb S, of which molecules are organized into polymeric beams when deoxygenated and give the RBC an elongated and rigid conformation, called a ‘‘sickle-shaped red blood cell’’. After the sickling process, the red blood cells begin to show changes in membrane proteins and increased expression of adhesion molecules that, consequently, lead to red blood cell adhesion to the endothelium. This process triggers an inflammatory phenomenon, activation of coagulation, hypoxia, ischemia and local infarction (Glassberg, 2011).
which may block different areas of the microcirculation which result in organs dysfunction including endocrine organs, erectile dysfunction, osteoporosis, thyroid dysfunction and gonadal failure, because 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 males 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 females with sickle cell anemia(Smith-Whitley et al., 2014).

Considering these facts, this study aimed to carry out an integrative literature review to analyze the frequency of Anti-mullerian hormone (AMH) levels and its consequences in females with sickle-cell anemia. That AMH is considered as a newer marker for ovarian function (Visser et al., 2006), in addition AMH plays crucial roles in sexual differentiation and gonadal function (Ireneet al., 2016), because AMH regulate gonadotropin gene expression (Bedecarrats et al., 2003), and production of sex hormones.

1.2. Literature review

1.2.1. Sickle cell disease

Sickle cell disease (SCD) is an inherited autosomal recessive hemoglobinopathy; homozygous sickle cell anemia (Hb SS) is the most common, while the doubly heterozygote conditions of Hb SC and Hb S-Beta-thalassemia also cause sickling disease (Hoffbrand, 2006).

Female with sickle cell anemia with iron overload may have gonadal hormone deficiency recommendations include regular iron chelation for prevention of irreversible damage of the ovaries and attaining normal sexual maturation and regular follow up for females with assessment of puberty as they are more vulnerable to develop hypogonadism and may require hormonal replacement therapy(Hagag et al., 2016).

1.2.1.1. Genetics of Sickle cell Disease

Normally, humans have hemoglobin A, which consists of two alpha and two beta chains, hemoglobin A2, which consists of two alpha and two delta chains, and hemoglobin F, consisting of two alpha and two gamma chains in their bodies. Of these, hemoglobin F dominates until about 6 weeks of age. Afterwards, hemoglobin A dominates throughout life.

Sickle-cell conditions have an autosomal recessive pattern of inheritance from parents. The types of hemoglobin a person makes in the red blood cells depend on what hemoglobin genes are inherited from her or his parents. If one parent has sickle-cell anemia and the other has sickle-cell trait, then the child has a 50% chance of having sickle-cell disease and a 50% chance of having sickle-cell trait. When both parents have sickle-cell trait, a child has a 25% chance of sickle-cell disease, 25% do not carry any sickle-cell alleles, and 50% have the heterozygous condition.

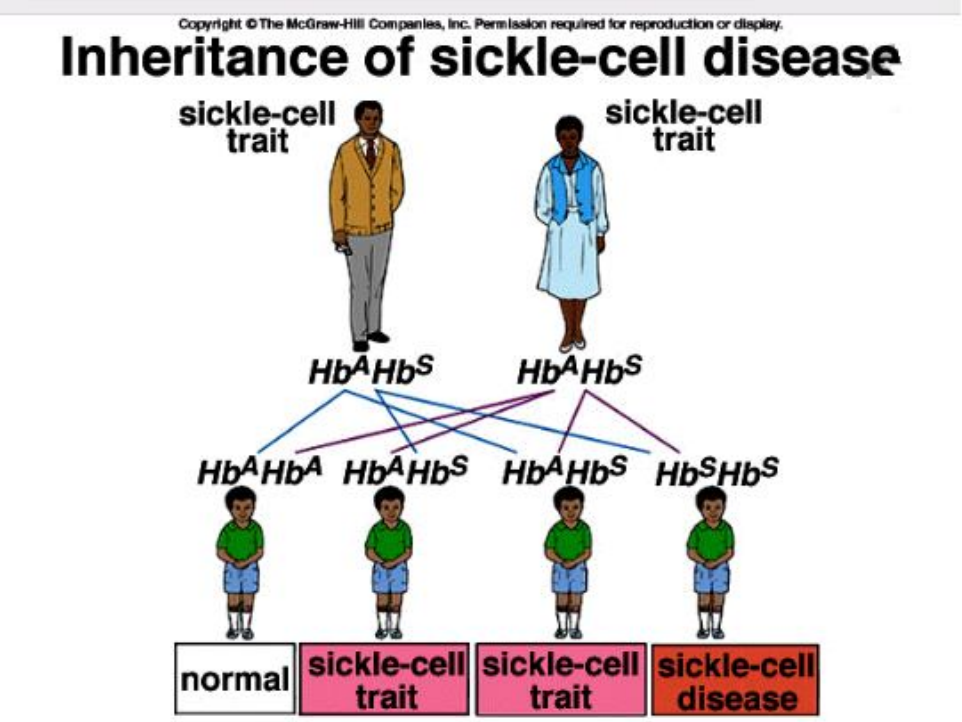

Figure 1.1: Inheritance of sickle cell disease.

In people heterozygous for Hb S (carriers of sickling hemoglobin), the polymerization problems are minor, because the normal allele is able to produce over 50% of the hemoglobin. In people homozygous for Hb S, the presence of long-chain polymers of Hb S distort the shape of the red blood cell from a smooth doughnut-like shape to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries. The sickle-cell disease occurs when the sixth amino acid, glutamic acid, is replaced by valine to change its structure and function; as such, sickle-cell anemia is also known as E6V. valine is hydrophobic, causing the hemoglobin to collapse on it occasionally. The structure is not changed otherwise. When enough hemoglobin collapses on itself the red blood cells become sickle-shaped (Allison, 2009).

The gene defect is a known mutation of a single nucleotide (A to T) of the β-globin gene, which results in glutamic acid (E/Glu) being substituted by valine (V/Val) at position 6. Note, historic numbering put this glutamic acid residue at position 6 due to skipping the methionine (M/Met) start codon in protein amino acid position numbering. Current nomenclature calls for counting

the methionine as the first amino acid, resulting in the glutamic acid residue falling at position 7. Many references still refer to position 6 and both should likely be referenced for clarity. Hemoglobin S with this mutation is referred to as Hb S, as opposed to the normal adult Hb A. The genetic disorder is due to the mutation of a single nucleotide, from a GAG to GTG codon on the coding strand, which is transcribed from the template strand into a GUG codon. Based on genetic code, GAG codon translates to glutamic acid (E/Glu) while GUG codon translates to valine (V/Val) amino acid at position 6. This is normally a benign mutation, causing no apparent effects on the secondary, tertiary, or quaternary structures of hemoglobin in conditions of normal oxygen concentration. What it does allow for, under conditions of low oxygen concentration, is the polymerization of the Hb S itself. The de-oxy form of hemoglobin exposes a hydrophobic patch on the protein between the E and F helices. The hydrophobic side chain of the valine residue at position 6 of the beta chain in hemoglobin is able to associate with the hydrophobic patch, causing hemoglobin S molecules to aggregate and form fibrous precipitates (Allison, 2009).

The allele responsible for sickle-cell anemia can be found on the short arm of chromosome 11, more specifically 11p15.5. A person who receives the defective gene from both father and mother develops the disease; a person who receives one defective and one healthy allele remains healthy, but can pass on the disease and is known as a carrier or heterozygote. Heterozygotes are still able to contract malaria, but their symptoms are generally less severe (Allison, 2009).

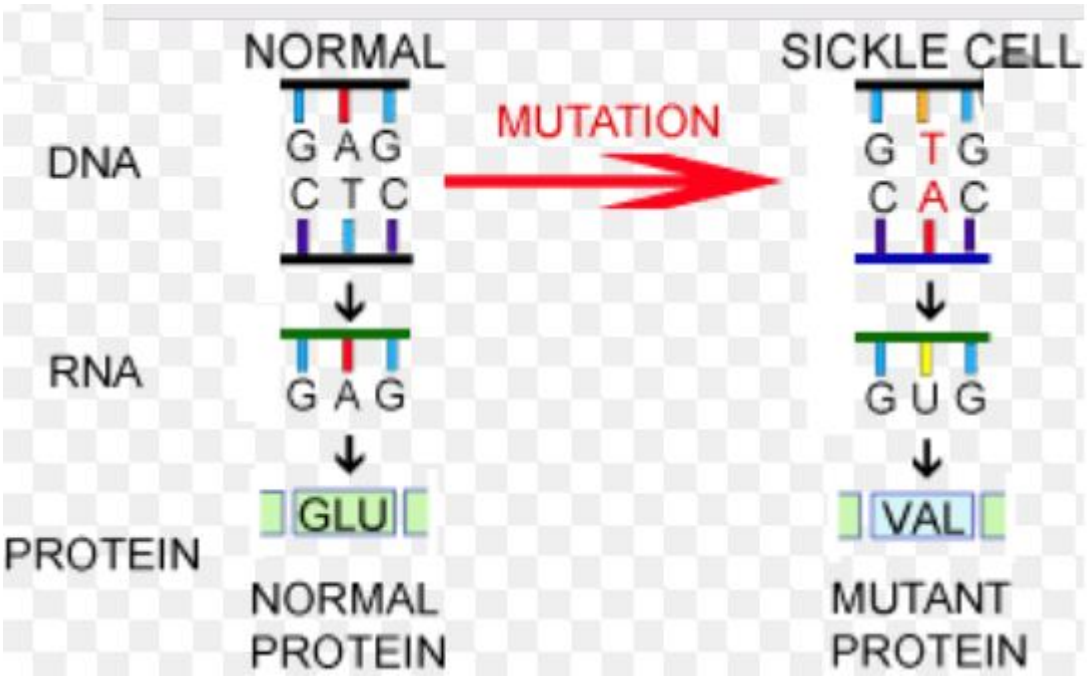

Figure 1.2: The mutation of genetic disorder of sickle cell disease

1.2.1.2. Clinical features of sickle cell anemia:

Clinical features of sickle cell anemia arecharacterized by severe hemolytic anemia punctuated by crises. The symptoms of anemia are often mild in relation to the severity of the anemia because Hb S gives up oxygen to tissues relatively easily compared with Hb A, its O2 dissociation curve being shifted to right. The clinical expression of Hb SS is very variable; some patients having an almost normal life, free of crises but others develop sever crises even as infant and may die in early childhood or as young adults. Crises may be vaso-occlusive, visceral, aplastic or hemolytic.

• Painful vaso-occlusive crises: are the most frequent and precipitated by such factors as infection, acidosis, dehydration or de-oxygenation (e.g: altitude, operations, obstetric delivery, stasis of the circulation, exposure to cold, violent exercise, etc...), infarcts may occur in a variety of organs including the bones (hips, shoulders and vertebrae are commonly affected) the hand-foot syndrome is painful dactylitis (swollen finger) caused by infarcts of small bones, it is

frequently the first presentation of the disease and may lead to digits of varying length. Infarct also occurs in lungs and spleen. The most serious vaso-occlusive crisis is of the brain (a stroke occurs in 7% of all patients) or spinal cord.

• Visceral sequestration crises: caused by sickling within organs and pooling of blood , often with a severe exacerbation of anemia, the acute sickle chest syndrome is most common cause of death after puberty, it presents with dyspnea, falling Po2,chest pain and pulmonary infiltration chest X-ray, treatment is with analgesia, oxygen, exchange transfusion and ventilator support if necessary. Hepatic and girdle sequestration crises and splenic sequestration all may lead to severe illness requiring exchange transfusion. Splenic sequestration is typically seen in infants and present with an enlarging spleen, falling hemoglobin and abdominal pain, treatment is with transfusion and patients must be monitored at regular intervals as progression may be rapid, attacks tend to be recurrent and splenectomy is often advised (Hoffbrand, 2006).

• Aplastic crises: these may occur as a result of infection with parvovirus or folate deficiency and are characterized by a sudden fall in hemoglobin, usually requiring transfusion. They are characterized by a fall in reticulocytes as well as hemoglobin.

• Hemolytic crises: these are characterized by an increase rate of hemolysis with a fall in hemoglobin but rise in reticulocytosis and usually accompany painful crises (Hoffbrand, 2006).

Other clinical features:

• Ulcers of the lower legs are common, as a result of vascular stasis and local ischemia.

• The spleen is enlarged in infancy and early childhood but later is often reduced in size as a result of infarcts (autosplenectomy).

• A proliferative retinopathy and priapism are other clinical complications.

• Chronic damage to the liver may occur through microinfarcts.

• Pigment (bilirubin) gall stones are frequent.

• The kidneys are vulnerable to infarctions of the medulla with papillary necrosis.

• Failure to concentrate urine aggravates the tendency to dehydration and crises, and nocturnal enuresis is common.

• Osteomyelitis may also occur, usually from Salmonella spp (Hoffbrand, 2006).

1.2.1.3. Laboratory diagnosis of sickle cell anemia:

The hemoglobin is usually 6-9 g\dl low in comparison to symptoms of anemia. Sickle cells and target cells occur in the blood. Features of splenic atrophy (e.g: Howell-Jolly bodies) may also be present. Screening tests for sickling are positive when the blood is deoxygenated (e.g. with dithionate and Na2HPO4). Hemoglobin electrophoresis in Hb SS, no Hb A is detected. The amount of Hb F is variable and is usually 5-15 %; larger amounts are normally associated with a milder disorder (Hoffbrand, 2006).

1.2.1.4. Treatment & Management of sickle cell anemia:

• **Prophylactic**: avoid those factors known to precipitate crises, especially dehydration, anoxia, infections, stasis of the circulation and cooling of the skin surface.

• **Folic acid**: 5 mg\day.

• Good general nutrition and hygiene.

• Pneumococcal, hemophilus and meningococcal **vaccination** and regular oral penicillin are effective at reducing the infection rate with these organisms.

• **Oral penicillin** should start at diagnosis and continue at until puberty.

• **Hepatitis B vaccination** is also given as transfusions may be needed.

• Crises- treat by **rest, warmth, rehydration** by oral fluids and intravenous normal saline and antibiotics if infection is present.

• **Analgesia** at the appropriate level should be given; suitable drugs are paracetamol, a non-steroidal anti-inflammatory agent and opiates, e.g. continuous subcutaneous diamorphine.

• **Blood transfusion** is given only if there is severe anemia with symptoms.

• **Exchange transfusion** may be needed particulary if there is neurological damage, a visceral sequestration crises or repeated painful crises; this is aimed at achieving an Hb S percentage of less than 30 in severe cases. Also transfusions with normal blood is needed for pregnancy to reduce Hb S levels, and for anesthesia to avoid hypoxemia or acidosis, sometimes it given repeatedly as prophylaxis to patients having frequent crises or organ damage e.g. brain damage to suppress Hb S production over a period of several months or years, but iron overload and alloimmunization against donated blood are common problems (Hoffbrand, 2006).

• **Hydroxyurea**(15-20 mg/kg) can increase Hb F levels and has been shown to improve the clinical course of patients who are having three or more painful crises each year; it should not be used during pregnancy; hydroxyurea belongs to a class of compounds called hydroxamic acids, which can bind metals, the primary cytotoxic effect of hydroxyurea lies in its ability to inhibit ribonucleotide reductase by binding the reductase's two iron molecules and inactivating a critical tyrosyl radical, this cytotoxic effect of hydroxyurea reduces the production of red cells containing a high level of sickle hemoglobin, which tend to arise from rapidly dividing precursors, and favors the production of red cells containing a high fetal hemoglobin level (F cells), which arise from progenitors that divide less rapidly, this drug also reduces the numbers of white cells and platelets, potentially reducing their roles in vascular injury. Another potentially important effect of hydroxyurea is that metabolism of the drug results in the production of nitric oxide, Soluble guanylate cyclase, an enzyme containing heme iron, is stimulated by nitric oxide, a reaction that results in the production of fetal hemoglobin, as shown in vitro, the production of nitric oxide may also compensate for the loss of endogenous nitric oxide due to intravascular hemolysis, hydroxyurea should not be given to patients with severe hypoplastic anemia, leukopenia, or thrombocytopenia, it should not be given during pregnancy or breast-feeding, and both men and women who are taking it should use contraception, since this agent is considered to be a teratogen (Platt,2008). The Committee recommends classification according to regulation (EC) 1272/2008 of the European Union, for hydroxyurea, these recommendations are: - for effects on fertility, the European recommends classifying hydroxyurea in category 1B (presumed human reproductive toxicant) and labelling with H360F (may damage fertility). -For effects on development, the Committee recommends classifying hydroxyurea in category 1B (presumed human reproductive toxicant) and labelling with H360D (may damage the unborn child) (Agrawal et al., 2014).

• **Stem cell transplantation** can cure the disease and many patients have now been successfully treated. The mortality rate is less than 10%. Transplantation isonly indicated in the severest of cases whose quality of life or lifeexpectancy are substantially impaired. Research into other drugs, e.g. butyrates, to enhance Hb F synthesis or to increase the solubility of Hb S is taking place. Gene therapy is a distant prospect not yet available (Hoffbrand, 2006).

• **Omega-3** is a new treatment which has an effective role in fertility by its main role in regulating hormones, increase blood flow to the uterus, reduce sensitivity to the hormone prolactin which can suppress ovulation, increase cervical mucus, improving reproductive cell structure and improving egg quality which result in prolonging the females reproductive lifespan , ovarian reserve and function. (Deepikaet al., 2012) .

1.2.1.5. Sickle celltrait:

This is a benign condition with no anemia and normal appearance of red cells on a blood film; hematuria is the most common symptom and is thought to be caused by minor infarcts of the renal papillae. Hb S varies from 25-45% of the total hemoglobin. Care must be taken with anesthesia, pregnancy and at high altitudes. A summary of the risks associated with sickle cell trait is as follows :

• Isothenuria with loss of maximal renal concentrating ability(Gupta et al., 1991).

• Hematuria secondary to renal papillary necrosis (Heller et al., 1979).

• Sudden idiopathic death with exercise (Steinberg, 1999).

• Glaucoma or recurrent hyphema following a first episode of hyphema (Sears et al., 1978).

• Renal medullary carcinoma in young people (ages 11 to 39 years) (Davis et al., 1995).

• Early onset of end stage renal disease from autosomal dominant polycystickidney disease(Yium et al., 1994).

1.2.1.6. Complications of Sickle cell anemia:

Sickle-cell anaemia can lead to various complications, including:

Increased risk of severe bacterial infections due to loss of functioning spleen tissue. These infections are typically caused by encapsulated organisms such as Streptococcus pneumoniae and Haemophilus influenzae. Daily penicillin prophylaxis is the most commonly used treatment during childhood, with some haematologists continuing treatment indefinitely. Patients benefit today from routine vaccination for S. pneumoniae (Kavanagh et al., 2011).

Stroke, which can result from a progressive narrowing of blood vessels, prevents oxygen from reaching the brain. Cerebral infarction occurs in children and cerebral haemorrhage in adults.

Silent stroke causes no immediate symptoms, but is associated with damage to the brain. Silent stroke is probably five times as common as symptomatic stroke. About 10–15% of children with SCD suffer strokes, with silent strokes predominating in the younger patients (Adams, 2007).

Cholelithiasis (gallstones) and cholecystitis may result from excessive bilirubin production and precipitation due to prolonged haemolysis.

Osteomyelitis (bacterial bone infection), the most common cause of osteomyelitis in SCD is Salmonella (especially the atypical serotypes Salmonella typhimurium, Salmonella enteritidis, Salmonella choleraesuis and Salmonella paratyphi B), followed by Staphylococcus aureus and Gram-negative enteric bacilli perhaps because intravascular sickling of the bowel leads to patchy ischaemic infarction (Almeida andRoberts, 2005).

Opioid tolerance can occur as a normal, physiologic response to the therapeutic use of opiates. Addiction to opiates occurs no more commonly among individuals with sickle-cell disease than among other individuals treated with opiates for other reasons.

In eyes, background retinopathy, proliferative retinopathy, vitreous haemorrhages, and retinal detachments can result in blindness Regular annual eye checks are recommended (Elagouz et al., 2010).

During pregnancy, intrauterine growth retardation, spontaneous abortion, and pre-eclampsia and Chronic pain: Even in the absence of acute vaso-occlusive pain, many patients have unreported chronic pain (Smith et al., 2008).

Pulmonary hypertension (increased pressure on the pulmonary artery) can lead to strain on the right ventricle and a risk of heart failure; typical symptoms are shortness of breath, decreased exercise tolerance, and episodes of syncope 21% of children and 30% of adults have evidence of pulmonary hypertension when tested; this is associated with reduced walking distance and increased mortality (Caughey et al., 2015).

Chronic kidney failure due to sickle-cell nephropathy manifests itself with hypertension, protein loss in the urine, loss of red blood cells in urine and worsened anaemia. If it progresses to end-stage renal failure, it carries a poor prognosis (Powars et al.,1991).

1.2.1.7. Epidemiology of sickle cell anemia:

Internationally The highest frequency of sickle cell disease is found in tropical regions, particularly sub-Saharan Africa, tribal regions of India and the Middle-East (Weatherall and Clegg, 2001). Migration of substantial populations from these high prevalence areas to low prevalence countries in Europe has dramatically increased in recent decades and in some European countries sickle-cell disease has now overtaken more familiar genetic conditions such as haemophilia and cystic fibrosis (Roberts and Montalembert, 2007).

Localythe prevalence of SC disease frequencies in different areas of Sudan ranging from 0.8% in central Sudan to 30.4% in Western Sudan among Messeryia tribe (a branch of the Baggara tribes) in Darfur and Messeryia Hummer of Kordofan showed the highest rate of sickle cell disease, also disease concentrated in two tribes, Bederia and Fulani 18%.Many indigenous tribes that inhabit Darfur region and belong to the Negroid ethnic group and are a part of Nilo-Saharan language family such as the Berge, Fur and Masaleet had the highest frequencies of the S gene among them, Northern Sudan and Eastern Sudan show very low prevelance of disease (Elderdery et al., 2011; Bayoumi et al., 1995).

1.2.2. Anti-Mullerian hormone:

Anti-mullerian hormone AMH is a protein that in humans is encoded by the AMH gene (Cate et al., 1986). It inhibits the development of the mullerian ducts “paramesonephric ducts” in male embryo (Berhenger, 1994).

It has also been called Mullerian inhibiting factor (MIF), Mullerian inhibiting hormone (MIH), Mullerian inhibiting substance (MIS) and Anti paramesonephric hormone (APH), Expression of AMH is activated by SOX9 transcription factor in the male sertoli cells and causes the irreversible regression of the Mullerian duct, in the absence of AMH, the Müllerian ducts develop into the uterus, fallopian tubes and the upper part of the vagina (Munsterberg and Lovell, 1991), because AMH expression is critical to sex differentiation at a specific time during

fetal development it appears to be tightly regulated by SF1, GATA factors, DAX1 and FSH. Mutations in both the AMH gene and type 2 AMH receptors have been shown to cause the persistence of mullerian derivatives in males that are otherwise normally virilized.

AMH expression also occurs in ovarian granulosa cells of females postpartum and serves as a molecular biomarker for relative size of the ovarian reserve. In humans the number of cells in the follicular reserve can be used predict timing of menopause, In bovine, AMH can be used for selection of females in multi-ovulatory embryo transfer programs by predicting the number of antral follicles developed to ovulation, in the ovaries of female fetuses, AMH expression has been observed as early as 32 weeks gestation in humans (Rajpert et al., 1999), in primordial follicles, AMH expression seems to be absent, AMH immunostaining can first be observed in granulosa cells of follicles at the primary stage of development. In one study, 75% of secondary follicles were positive for AMH immunostaining, the strongest staining was observed in pre-antral and small antral follicles (Weenen et al., 2004).

AMH continues to be expressed in the growing follicles in the ovary until they have reached the size and differentiation state at which they may be selected for dominance, in the mouse this occurs at the early antral stage in small growing follicles (Durlinger et al., 2002), whereas in the human it is evident in antral follicles 4–6 mm in diameter (Weenen et al., 2004),thus, AMH is expressed in follicles that have undergone recruitment from the primordial follicle pool but have not been selected for dominance, AMH is not expressed in atretic follicles or theca cells (Ueno et al., 1989; Munsterberg and Lovell, 1991; Hirobe et al., 1994).

Although AMH has been shown to have mainly autocrine and paracrine actions in follicle development, the protein is also measurable in serum, antral follicles are considered to be the primary source of circulating AMH as they contain a large number of granulosa cells, a body of clinical data suggests that AMH is preferentially and constantly secreted by small rather than large antral follicles, the amount and the rate of AMH production by a single antral follicle should be investigated and in particular its modification in relation to the follicular hormonal milieu and to ageing.

Granulosa cells secrete AMH into both the bloodstream and follicular fluid, although concentrations are very much higher in the latter, however, the exact role of AMH in this

compartment has not been elucidated, in addition to paracrine and autocrine actions on the granulosa cells, a possible direct role in modulating oocyte physiology may be identified.

AMH type II receptors have been identified in tissues other than the ovary, such as human endometrium (Wang et al., 2009), and several human cancer cell lines, including those originating from the cervix (Barbie et al., 2003), endometrium (Renaud et al., 2005), ovarian epithelium (Masiakos et al., 1999; Ha et al., 2000) and breast (Segev et al.,2000).

As an early marker capable of identifying subtle damage to the ovaries, AMH may become the test of choice in studies on ovarian damage due to any kind of agent, such as chemotherapy, ovarian surgery and even disease processes like endometriosis studies on longitudinal changes in AMH levels during and after chemotherapy may also be very informative in establishing the gonadotoxicity of different chemotherapeutics and the extent of possible protective effect of oral contraceptive and GnRH analog co-treatment.

1.2.2.1. Structure of AMH:

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; it has a molar mass of 140 kDa. Genetically In humans, the gene for AMH is AMH on chromosome 19p13.3, while the gene AMHR2 codes for its receptor on chromosome 1 (Jackowiak, 1995).

1.2.2.2. Biological roles of AMH:

Embryogenesis: in mammals, AMH prevents the development of the Müllerian ducts into the uterus and other Müllerian structures, the effect is ipsilateral, that is each testis suppresses Müllerian development only on its own side, in humans, this action takes place during the first 8 weeks of gestation, if no hormone is produced from the gonads, the Müllerian ducts automatically develop, while the Wolffian ducts, which are responsible for male reproductive parts, automatically die. Amounts of AMH that are measurable in the blood vary by age and sex, AMH works by interacting with specific receptors on the surfaces of the cells of target tissues, the best-known and most specific effect, mediated through the AMH type II receptors, includes

programmed cell death (apoptosis) of the target tissue (the fetal Müllerian ducts) (Jackowiak, 1995).

Ovarian: AMH has important roles in postnatal ovarian function (Frank et al., 2008), is expressed by granulosa cells of the ovary during the reproductive years, and limits the formation of primary follicles by inhibiting excessive follicular recruitment by FSH. Some authorities suggest it is a measure of certain aspects of ovarian function, useful in assessing conditions such as polycystic ovary syndrome and premature ovarian failure, it is useful to predict a poor ovarian response in in vitro fertilization (IVF), but it does not appear to add any predictive information about success rates of an already established pregnancy after IVF, additionally, AMH levels are used to determine a women's remaining egg supply, However, the patterns of expression of AMH and its type II receptor in the postnatal ovary indicate that AMH may play an important role in ovarian folliculogenesis. This review describes several in vivo and in vitro studies showing that AMH participates in two critical selection points of follicle development: it inhibits the recruitment of primordial follicles into the pool of growing follicles and also decreases the responsiveness of growing follicles to FSH (Frank et al., 2008).

There is a strong correlation between AMH levels and number of primordial follicles ($r = 0.83$, $P < 0.0001$), so AMH is an excellent marker to assess the quantitative aspect of ovarian reserve, which may be useful for women at risk for early ovarian aging such assurvivors of childhood cancers (Kevenaar et al., 2006) and a marker of oocyte quality (Garcia et al., 2009).

Other functions: AMH production by the Sertoli cells of the testes remains high throughout childhood in males but declines to low levels during puberty and adult life, AMH has been shown toregulate production of sex hormones and changing AMH levels (rising in females, falling in males) may be involved in the onset of puberty in both sexes, Functional AMH receptors have also been found to be expressed on neurons in the brains of embryonic mice, and are thought to play a role in sexually dimorphic brain development and consequent development of gender-specific behaviors.

Regulation of gonadotropins gene express by mullerian inhibiting substance (Bedecarrats et al., 2003).

AMH act as a co-regulator of steroidogenesis in granulosa cells, as AMH levels appear to be related to estradiol levels in follicular fluid from small antral follicles (Andersen and Byskov, 2006), this was confirmed by a recent study which showed that polymorphisms in the gene for AMH or AMH receptor type II seem to be related to follicular phase estradiol levels, suggesting a role for AMH in the FSH-induced steroidogenesis in the human ovary (Kevenaar et al., 2007).

1.2.2.3. Blood levels of AMH:

In healthy females AMH levels are almost undetectable at birth, after an initially slight increase in the weeks after birth, AMH levels increase peaking during late puberty (Lee et al., 1996) and then show a progressive decline throughout reproductive life as the follicular reserve becomes depleted (Lee et al., 1996; Guibourdenche et al., 2003), and finally becoming undetectable after menopause (Van Rooij et al., 2004 ; La Marca et al., 2005).

AMH may constitute a unique endocrine parameter for the investigation of ovarian function, since several studies have demonstrated that, in contrast to sex steroids, gonadotrophins and peptides such as inhibin B, AMH serum levels do not significantly change throughout the menstrual cycle (Hehenkamp et al., 2006 ; La Marca et al., 2006 ; Tsepelidis et al., 2007; Streuli et al., 2008), however, others have reported significant cyclical fluctuations in AMH levels with a rapid decrease in AMH levels in the early luteal phase (Wunder et al., 2008; Streuli et al., 2009). Excursions from mean levels of +3 to −19%, have been reported (Wunder et al., 2008; Streuli et al., 2009), these variations are similar to reported intercycle fluctuations for AMH (Streuli et al., 2008), in the clinical setting the inter- and intra-cycle variability in serum AMH levels may be considered to be low enough to permit random timing of AMH measurements during the menstrual cycle, Of course, further studies on a large sample of patients, based on daily blood samples are needed to clarify whether AMH levels vary significantly during the menstrual cycle, up to now, reported fluctuations appear to be of small amplitude, and therefore probably of minor significance when interpreting data for clinical purposes. Furthermore, AMH levels appear to be unmodified in conditions under which endogenous gonadotrophin release is substantially diminished, such as during pregnancy (La Marca et al., 2005), under GnRH agonist pituitary down-regulation (Mohamed et al., 2006) and oral contraceptive administration (Arbo et al., 2007; Somunkiran et al., 2007; (Streuli et al., 2008), this indicates that non-cyclic FSH-independent ovarian activity persists even when pituitary FSH secretion is

suppressed, these findings are consistent with the concept that AMH levels reflect the continuous FSH-independent non-cyclic growth of small follicles in the ovary.

Also AMH levels decrease under current use of oral contraceptives and current tobacco smoking.

1.2.2.4. Clinical significant:

The role of AMH as a marker for ovarian reserve: the age-related decline in female reproductive function due to the reduction of the ovarian follicle pool and the quality of oocytes has been well established (Macklon and Fauser, 2005), a reliable marker for the age at which subfertility will occur would have great potential value as a predictor of future reproductive lifespan, the ideal marker would show a significant change in levels from adolescence to the late reproductive period, increased basal levels of FSH, and a decrease in inhibin B and in the antral follicle count (AFC) on ultrasound examination are widely taken to indicate a reduced ovarian reserve (Broekman's et al., 2006).

That AMH is used as a marker for diminished ovarian reserve (DOR) in female with sickle cell anemia were treated by hydroxyurea (Elchurietal., 2015).

1.3. Rationale

As medical advances improve survival, reduce disease-related morbidity and improve quality of life, reproductive issues will take higher priority in sickle cell disease community, because every hundreds of thousands around world die from diseases caused by anemia.

Etiologies of impaired male fertility are multifactorial but much less are known about the prevalence and etiologies of infertility in females with SCD.

Number of researches indicated that sickle cell anemia has numerous immediate health effects on gonadal functions, body weight and sexual maturation.

No studies were found in evaluation of Anti-Mullerian Hormone AMH in Sudanese females with sickle cell anemia that is why we attempt to evaluate levels of AMH in Sudanese females with sickle cell anemia.

1.4. Objectives

1.4.1. General objective:

To study the levels of Anti-mullerian hormone AMH among Sudanese females with sickle cell anemia.

1.4.2. Specific objectives:

1/ To measure serum AMH levels in Sudanese females with (sickle cell anemia and sickle cell trait) in comparison to healthy individuals.

2/ To correlate between levels of AMH, age and duration of treatment among Sudanese females with sickle cell anemia.

Chapter Two

Materials and methods

2. Materials and Methods

2.1. Materials

2.1.1. Study design:

This is a descriptive analytical case control study.

2.1.2. Study area:

The study was conducted in sickle cell anemic Sudanese female in JaaferEbnOuf hospital in Khartoum state and Hejleej hospital in western Kordofan state.

2.1.3. Study period:

The study took a period from March to December 2016.

2.1.4. Study population:

This study included 50 Sudanese sickle cell anemic females and 30 healthy individuals as control. Age was matched, ranged from (2 to 38) years old.

2.1.5. Inclusion criteria:

Sudanese female with sickle cell anemia were included.

2.1.6. Exclusion criteria:

Icterus 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 AMH were excluded.

2.1.7.Ethical consideration:

About 2ml of venous blood was collected from each patient at the plane containers, after clotting, centrifuged for 3 minutes at 3000 RPM to obtain serum and analyzed.

2.1.8. Sample size and sampling technique:

Individuals who voluntarily accepted to participate in the study were enrolled. Data was collected by using questionnaire (Appendix 1).

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2.2. Methods

Estimation of serum AMH concentration by using Mindary MR-96A auto analyzer.

Principle of method: The Fortress AMH ELISA assay used a monoclonal antibody and an AMH HRP conjugate in an anti AMH coated plate. After incubation and subsequent washing the wells were incubated with a substrate solution. Measurement was performed after stopping the reaction used an acidic stop solution (Appendix 2).

2.2.1. 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.

2.2.2. Data analysis:

Data was analyzed by using the SPSS computer program.

Chapter Three

Results

Fifty Sudanese females with sickle cell disease were enrolled in this study to assess the levels of Anti-Mullerian Hormone(AMH)and thirty Sudanese females without sickle cell disease were served as control group.

Mindary MR-96A auto analyzer was used for estimation of AMH levels.

Statistical analysis was done by using SPSS computer program and the results were as follow:

- **Table (3.1)** shows the comparison between means of serum AMH levels in Sudanese females with sickle cell disease and non-sickle cell disease.
- **Table (3.2)** shows the comparison between means of serum AMH levels in Sudanese females with sickle cell disease, sickle cell trait and non-sickle cell disease.
- **Figure (3.1)** a scatter plot shows no correlation between serum AMH and age among Sudanese females with sickle cell anemia (p.value=0.993 r=0.001).
- **Figure (3.2)** a scatter plot shows no correlation between serum AMH and duration of hydroxyurea treatment among Sudanese females with sickle cell anemia (p.value=0.510, r=0.185).

Table (3.1): Comparison between means concentration of serum AMH in Sudanese females with sickle cell disease and non-sickle cell disease.

Groups	AMH concentration (ng/ml) Mean ± SD	P.value
Females with Sickle cell disease.	0.697±0.449	0.862
Non-sickle cell disease females.	0.684±0.226	

Independent sample T test was used for comparison, value consider significant at level ≤ 0.05

Table (3.2): Comparison between means concentration of serum AMH in Sudanese females with sickle cell disease, sickle cell trait and non-sickle cell disease.

Groups	AMH concentration (ng/ml) Mean ± SD	p-*value*
Females with sickle cell disease	0.691 ± 0.499	0.997
Sickle cell trait	0.720 ± 0.192	0.963
Non-sickle cell disease	0.684 ± 0.226	

One-Way Anova test was used for comparison, value consider significant at level ≤ 0.05

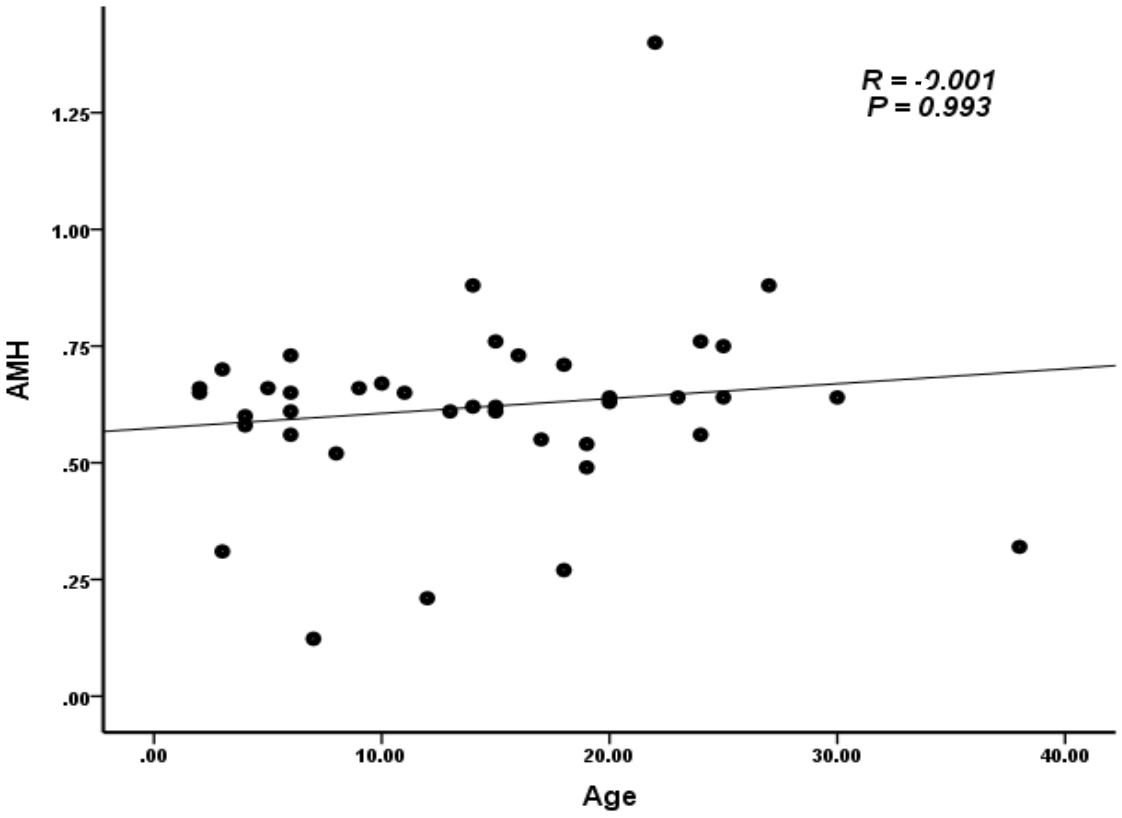

Figure (3.1): Correlation between serum AMH levels (ng\ml) and age among Sudanese females with sickle cell anemia.

(p.value=0.993 r=0.001).

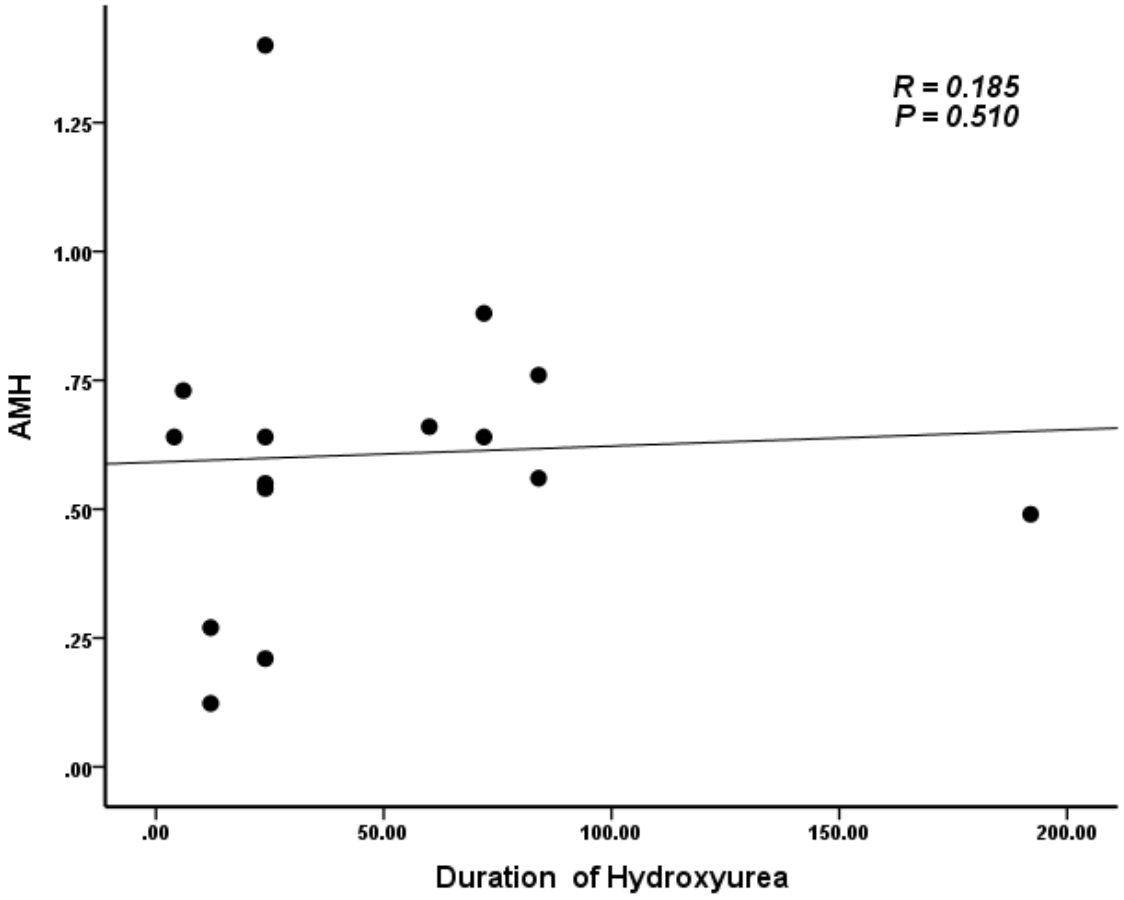

Figure (3.2): Correlation between serum AMH levels (ng\ml) and duration of hydroxyurea treatment (days) among Sudanese females with sickle cell anemia. (p.value=0.510, r=0.185).

Chapter Four

Discussion, conclusion and recommendations

4. Discussion, conclusion and recommendations

4.1. Discussion

Gonadal hypofunction is described in male and female patients with sickle cell anemia (El-Hazmi et al., 1992).

Females with sickle cell anemia with iron overload may have gonadal hormone deficiency if untreated may develop hypogonadism (Hagag et al., 2016).

Accordingly this is a case control study aimed to study the level of Anti-mullerian hormone (AMH) among Sudanese females sickle cell anemia. Eighty Sudanese females (50 female with sickle cell disease and 30 healthy females without sickle cell disease) were enrolled in this study.

After evaluation of serum AMH by Mindary MR-96A autoanalyzer, the statistical analysis was done by using SPSS computer program, the results showed that serum AMH was insignificantly different in mean concentration of serum AMH of females with sickle cell disease when compared to females without sickle cell disease, with p.value of (0.862) and Mean ± SD of(0.697±0.449) VS (0.684±0.226).

This result disagreed with result of study done by Elchuries and his team to show the effects of hydroxyurea and bone marrow transplant on AMH level in female with sickle cell anemia (SCA), showed that AMH level in sickle cell anemic patients who treated by hydroxyurea was < 5^{th} percentile for age-matched controls which defined as diminished ovarian reserve (DOR) (Elchuri et al., 2015).

The contradict finding might be justified by doses of new treatment called omega3 which was taken continuously with folic acid by patients who enrolled in our study, which has an effective role in prolonging the female reproductive lifespan, improving egg quality and reproductive structure cells (Deepika et al., 2012), so in our study there was no significant difference of serum AMH in sickle cell disease females compared to control that AMH is a marker for ovarian reserve and ovarian function (van et al., 2002) and oocyte quality (Garcia et al., 2009).

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 insignificant differences in means concentration of AMH between sickle cell disease, sickle cell trait and control groups withp-value of (0.997 and 0.963) respectively, which indicates that the sickle cell disease does not affect on the AMH levels.

Person's correlation results showed that, there was no correlation between the AMH concentration and age with (p.value=0.993 r=0.001).

Also the results showed that there were no correlation between the AMH concentration and duration of hydroxyurea treatment with (p.value=0.510, r=0.185).

4.2. Conclusion

The study results concluded that:

• There was no difference between levels of AMH in Sudanese females with sickle cell disease and healthy individuals.

• There was no difference between AMH concentration in Sudanese females with sickle cell anemiaand sickle cell trait in comparison to healthy individuals.

• There were no correlation between serum AMH, age and duration of hydroxyurea treatment among Sudanese females with sickle cell anemia and in comparison to healthy individuals.

4.3. Recommendations

• AMH should be carefully evaluated in males and females with sickle-cell anemia to avoid complications of hypogonadism, sterility and infertility. That it consideras an early marker capable of identifying subtle damage to the ovaries due to any kind of agents.

• Further studies should be done to evaluate levels of vitamin D, Ferritin, Zinc and Selenium which may be disturbed according to disturbance of AMH level.

• Measurement of FSH, Inhibin B and Antral Follicle Count (AFC) on ultrasound examination are recommended with AMH as they are widely taken as sensitive markers profile for the ovarian reserve and function.

• Also the study recommended Omega-3-6-9 complex fatty acids in addition to folic acid protocol supplements continuously, with nutrition rich of Zinc, Selenium, vitamin A, E & C for females with sickle cell anemia who received hydroxyurea doses, to decrease hydroxyurea side effects on reproductive system, improve reproductive cell structure, egg quality andprolong the female's reproductive lifespan.

• Recently, there are systematic epidemiological studies to assess the prevalence rates of SCD and SCT in different areas in Sudan. So awareness to these tribes by estimating all gonadal hormones, minerals, vitamins and follow up is recommended. Because when reproductive issues take higher priority in sickle cell disease community; improve survival, reduce morbidity and improve quality of life will definitely occur.

References

References

Adams, R.J. (2007). Big strokes in small persons. Archives of Neurology. 64(11), 1567–74. PMID: 17998439..

Agrawal, R.K., Patel, R.K., Shah, V. & Trivedi B. (2014). Hydroxyurea in sickle cell disease. Drug Review. 30(2), 91-96.

Allison, A.C. (2009). Genetic control of resistance to human malaria. Current Opinion in Immunology. 21(5), 499–505.

Almeida, A.& Roberts, I. (2005). Bone involvement in sickle cell disease. British Journal of Haematology. 129(4), 482–90.

Andersen, C.Y. &Byskov, A.G. (2006). Estradiol and regulation of anti-Müllerian hormone, inhibin-A, and inhibin-B secretion: analysis of small antral and preovulatory human follicles' fluid. Journal of Clinical Endocrinology Metabolism. 91(10), 4064–69.

Arbo, E., Vetori, D.V., Jimenez, M.F., Freitas, F.M., Lemos, N. & Cunha- Filho, J.S. (2007). Serum anti-mullerian hormone levels and follicular cohort characteristics after pituitary suppression in the late luteal phase with oral contraceptive pills. Human reproduction. 22(12), 3192-3196.

Barbie, T.U., Barbie, D.A., MacLaughlin, D.T., Maheswaran, S. &Donahoe, P.K. (2003). Mullerian inhibiting substance inhibits cervical cancer cell growth via a pathway involving p130 and p107. Proceeding of the National Academy of Science of USA. 100(26), 15601–606.

Bayoumi, R. A., Taha, T.S. &Saha, N. (1995). A study of some genetic characteristics of the Fur and Baggara tribes of the Sudan. American Journal of Physical Anthropology. 67(4), 363-70.

Bedecarrats, G.Y., O'Neill, F.H., Norwitz, E.R., Kaiser, U.B. & Teixeira, J. (2003). Regulation of gonadotropin gene expression by Mullerian inhibiting substance. PNAS. 100(16), 9348-53.

Behringer, R.R. (1994). The in vivo role of mullerian- inhibiting substance. Current topics in developmental Biology. 29,171-187.

Broekmans, F.J., Kwee, J., Hendrisk, D.J. &Mol, B.W. (2006). A systematic review of tests predicting ovarian reserve and IVF outcome. Human reproduction update. 12(6), 685-718.

Cate, R.L., Mattaliano, R.J., Hession, C., Tizard, R., Farber, N.M., Cheung, A., Ninfa, E.G., Frey, A.Z., Gash, D.J. & Chow, E.P. (1986). Isolation of the bovine and human genes for mullerian inhibiting substance and expression of the human gene in animal cell. Cell. 45(5), 685-98.

Caughey, M.C., Poole, C., Ataga, K.I. &Hinderliter, A.L. (2015). Estimated pulmonary artery systolic pressure and sickle cell disease: a meta-analysis and systematic review. British Journal of Haematology. 170(3), 416–24.

Davis, C.J., Mostofi, F.K. &Sesterhenn, I.A. (1995). Renal Medullary Carcinoma. The seventh sickle cell nephropathy. Am J SurgPathol. 19(1), 1-11

Deepika, N., Hau, D.L., Erica, M.F., Sarah J.C., Dori Woods, Yvonne, A.W., Amy, H.P., LankaiGuo, Scott, J.R., Jonathan, L.T., Bo, R.R. & Mark, P. (2012). Prolonging the female reproductive lifespan and improving egg quality with dietary omega-3 fatty acids. Aging cell. 11(6), 1046-1054.

Durlinger, A.L., Visser, J.A. &Themmen, A.P. (2002). Regulation of ovarian function: the role of anti-mullerian hormone. Reproduction. 124(5), 601-9.

Elagouz, M., Jyothi, S., Gupta, B. and Sivaprasad, S. (2010). Sickle cell disease and the eye: old and new concepts. Survey of Ophthalmology. 55 (4), 359–77.

Elchuri, S.V., Williamson, R.S., Clark, B.R., Haight, A.F., Spencer, J.B., Buchanan, I., Hassan, S.I., Brown, M.R., Mertens, A.C. & Meacham, L.R. (2015). The effects of hydroxyurea and bone marrow transplant on antimullerian hormone (AMH) levels in females with sickle cell anemia. ELSEVIER. 55(1), 56-61.

Elderdery, A.Y., Mohamed, B.A., Cooper, A.J., Knight ,G. & Mills, J. (2011). Tribal distribution of haemoglobinopathies in a Sudanese patient population. Journal of Medical Laboratory and Diagnosis. 2(4), 31-37.

El-Hazmi, M.A., Bahakim, H.M, & Al-Fawazi, I. (1992). Endocrine function in sickle cell anemia patients. Journal of TropicalPeditarics. 38(6), 307-13.

Firth, P.G. &Head, C.A. (2004). Sickle cell disease and Anesthesia. Anesthesiology. 101(3), 766-785.

Frank, J., Broekmans, Visser, A.J., Joop, S., Leveva, E., Simone, L., Broer, Axel, P.N., Themmen, A.P., Bart, C. &Fauser, B.C. (2008). Antimullerian hormone and ovarian dysfunction. Trends in endocrinology and metabolism. 19(9): 340-347.

Garcia- Velasco, J.A., Motta, L., Rodriguez, S., Toribio, M., Martinez- Salazar, J. & Pacheco, A. (2009). Decreased concentrations of AMH in follicular fluid of women with endometrosis: A hypothetical new marker of oocyte quality. Jornal of Endometrosis and pelvic pain. 1(1), 52-56..

Glassber, J. (2011). Evidence- based management of sickle cell disease in the emergency department. Emergency Medicine Practice. 13(8), 1-20.

Guibourdenche, J., Lucidarme, N., Chevenne, D., Rigal, O., Nicolas, M., Luton, D., Léger, J., Porquet, D. & Noël, M. (2003). Anti-Müllerian hormone levels in serum from human foetuses and children: pattern and clinical interest. Mol Cell Endocrinol. 211(1-2), 55–63.

Gupta, A.K., Kirchner, K.A., Nicholson, R., Adams, J.G., Schechter, A.N., Noguchi, C.T. & Steinberg, M.H. (1991). Effects of a-thalassemia and sickle polymerization tendency on the urine-concentrating defect of individuals with sickle cell trait. Journal of Clinical Investigation. 88(6), 1963-8.

Ha, T.U., Segev, D.L., Barbie, D., Masiakos, P.T., Tran, T.T., Dombkowski, D., Glander, M., Clarke, T.R., Lorenzo, H.K., Donahoe, P.K., Maheswaran, S. (2000). Mullerian inhibiting substance inhibits ovarian cell growth through an Rb-independent mechanism. The Journal of Biological Chemistry. 275(47), 37101–37109.

Hagag, A.A., El-Farargy, M.S., Elrefaey, S. & Abo El-enein, A.M. (2016). Study of gonadal hormones in Egyptian female children with sickle cell anemia in correlation with iron overload. Hematology Oncology and Stem Cell Therapy. 9(1), 1-7.

Hehenkamp, W.J., Looman, C.W., Themmen, A.P., de Jong, F.H., TeVelde, E.R. &Broekmans, F.J. (2006). Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. The Journal Clinical of Endocrinology Metabolism. 91(10), 4057–4063.

Heller, P., Best, W.R., Nelson, R.B. &Becktel, J. (1979). Clinical implications of sickle cell trait and glucose-6-phosphate dehydrogenase deficiency in hospitalized black male patients. The New England Journal of Medicine. 300(18), 1001-5.

Hirobe, S., He, W.W., Gustafson, M.L., MacLaughlin, D.T. &Donahoe, P.K. (1994). Mullerian inhibiting substance gene expression in the cycling rat ovary correlates with recruited or graafian follicle selection. Biology of Reprodudtion. 50(6), 1238–43.

Hoffbrand, A.V., Moss, P.A. & Pettit, J.E. (2006). Gentic disorder of hemoglobin in: Essential heamatology 5th ed. Australia. Black well publishing. P. 85-91.

Irene, C., Filippo, C., Andrea M., Jyoti P., Soazik P., Sophie C., Francis C., Marc B. Didier D. Pascal P., Mel P., Rebecca C., Allan E.,Vincent P.& Paolo, G. (2016). Novel role for anti-mullerian hormone in regulation of GnRH neuron excitability and hormone secretion. Nature communications. 7(10055).

Jackowiak, J.T.,Szymankiewicz, W.A. &Trezeciak, W.H. (1995). Anti-mullerian hormone. Structure and role in sexual differentiation. Ginekol Pol. 66(1), 51-8.

Janse, F.,Donnez, J., Anckaert, E., De Jone, F.H., Fauser, B.C. & Dolmans, M.M. (2011). Value of ovarian function markers following orthotopic transplantation of ovarian tissue after gonadotoxic treatment. The Journal of Clinical Endocrinology and metabolism. 96(4), 1136-44.

Kavanagh, P.L.,Sprinz, P.G., Vinci, S.R., Bauchner, H. & Wang, C.J. (2011). Management of children with sickle cell disease: a comprehensive review of the literature. Pediatrics. 128(6), e1552–74.

Kevenaar, M.E.,Meerasahib, M.F., Karmer, P., Van de Lang-Born, B.M., De Jong, F.H., Groome, N.P., Themmen, A.P. &Visser. J.A. (2006). Serum anti mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology. 147(7), 3228-34.

Kevenaar, M.E.,Themmen, A.P., Laven, J.S., Sonntag, B., Fong, S.L., Uitterlinden, A.G., De Jong, F.H., Pols, H.A., Simoni, M. &Visser, J.A. (2007). Anti-Müllerian hormone and anti-Müllerian hormone type II receptor polymorphisms are associated with follicular phase estradiol levels in normo-ovulatory women. Human Reproductive. 22(6), 1547–54.

La Marca, A., De Leo, V., Giulini, S., Orvieto, R., Malmusi, S., Giannella, L. & Volpe, A. (2005a). AntiMullerian hormone in premenopausal women and after spontaneous or surgically induced menopause. J SocGynecolInvestig. 12(7), 545–548.

La Marca, A., Giulini, S., Orvieto, R., De Leo, V. & Volpe, A. (2005b). Anti-mullerian hormone concentrations in maternal serum during pregnancy. Human reproduction. 20(6), 1569-1572.

La Marca, A., Stabile, G., Artenisio, A.C. & Volpe, A. (2006). Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod. 21(12), 3103–3107.

Lee, M.M., Donahoe, P.K., Hasegawa, T., Silverman, B., Crist, G.B., Best, S., Hasegawa, Y., Noto, R.A., Schoenfeld, D. &MacLaughlin, D.T. (1996). Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J ClinEndocrinolMetab, 81(2), 571–576.

Macklon, N.S. &Fauser, B.C. (2005). Overian reserve. Seminars in Reproductive Medicine. 23(3), 248-56.

Masiakos, P.T., MacLaughlin, D.T., Maheswaran, S., Teixeira, J., Fuller, A.R. Jr., Shah, P.C., Kehas, D.J., Kenneally, M.K., Dombkowdki, D.M., Ha, T.U., Prefer, F.I. &Donahoe, P.K. (1999). Human ovarian cancer, cell lines, and primary ascites cells express the human Mullerian inhibiting substance (MIS) type II receptor, bind, and are responsive to MIS. Clinical Cancer Research. 5(11), 3488–99.

Muhamed, K.A., Davies, W.A. &Lashen, H. (2006). Anti-mullerian hormone and pituitary gland activity after prolonged down-regulation with goserelin acetate. Fertility and Sterility. 86(5), 1515-1517.

Munsterberg, A. & Lovell-Badage, R. (1991). Expression of the mouse anti-mullerian hormone gene suggests a role in both male and female sexual differentiation. Development. 113(2), 613-24.

Platt, O.S. (2008). Hydroxyurea for treatment of sickle cell anemia. The New England Journal of Medicine. 358(13), 1362-69.

Powers, D.R., Elliott- Mills, D.D., Chan, L. Niland, J., Hiti, A.L., Opas, L.M & Johnson C. (1991). Chronic renal failure in sickle cell disease: risk factors, clinical course and mortality. Annals of Internal Medicine. 115(8), 614-20.

Rajpert-De, M.E., Jorgensen, N., Gream, N., Muller, J., Cate, R.L. &Shakkebaek, N.E. (1999). Expression of anti-mullerian hormone during normal and pathological gonadal development: association with differentiation of sertoli and granulosa cell. The Journal of Clinical Endocrinology and Metabolism. 84(10), 3836-44.

Renaud, E.J., MacLaughlin, D.T., Oliva, E., Rueda, B.R. &Donahoe, P.K. (2005). Endometrial cancer is a receptor-mediated target for mullerian inhibiting substance. Proceedings of the National Academy of Sciences of USA. 102(1), 111–16.

Roberts, I. & De Montalembert, M. (2007). Sickle cell disease as a paradigm of immigration hematology: new challenges for hematologists in Europe. Haematologica. 92 (7), 865–71.

Sears, D.A. (1978).The morbidity of sickle cell trait: a review of the literature. American Journal of Medecine. 64(6), 1021-36.

Segev, D.L., Ha, T.U., Tran, T.T., Kenneally, M., Harkin, P., Jung, M., Maclaughlin, D.T., Donahoe, P.K. &Maheswaran, S. (2000). Mullerian inhibitingsubstance inhibits breast cancer cell growth through an NFkappaB-mediatedpathway. The Journal of Biological Chemistry. 275(37), 28371–28379.

Smith, W.R., Penberthy, L.T., Bovbjerg, V.E., McClish, D.K., Roberts, J.D., Dahman, B., Aisiku, I.P., Levenson, J.L. &Roseff, S.D. (2008). Daily assessment of pain in adults with sickle cell disease. Annals of Internal Medicine. 148 (2), 94–101.

Smith-Whitley, K. (2014). Reproductive issues in sickle cell disease. Blood. 124(24), 3538-43.

Somunkiran, A., Yavuz, T., Yucel, O. &Ozdemir, I. (2007). Anti-mullerian hormone levels during hormonal contraception in women with polycystic ovary syndrome. European Journal of Obstetrics & Gynecology and Reproductive Biology. 134(2), 196-201.

Steinberg, M.H. (1999). Management of Sickle cell disease. The New England Journal of Medecine. 340(13), 1021-30. PMID:10099145.

Streuli, I., Fraisse, T., Chapron, C., Bijaoui, G., Bischof, P. & De Ziegler, D. (2009). Clinical uses of anti-Müllerian hormone assays: pitfalls and promises. Fertility and Sterility. 91(1), 226-230.

Streuli, I.,Fraisse, T., Pillet, C., Lbecheole, V., Bischof, P. & De Ziegler, D. (2008). Serum anti-mullerian hormone levels remain stable throught the menstrual cycle and after oral or vaginal administration of synthetic sex steroids. Fertility and Sterility. 90(2), 395-400.

Tsepelidis, S., Devreker, F., Demeestere, I., Flahaut, A., Gervy, C.h. &Englert, Y. (2007). Stable serum levels of anti-Müllerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Human Reproductive. 22(7), 1837–1840.

Van Rooij, I.A.,Broekmans, F.J., TeVelde, E.R., Fauser, B.C., Bancsi, L.F., De Jong, F.H.&Themmen, A.P. (2002). Serum anti-mullerian hormone levels: a novel measure of ovarian reserve. Human reproductive. 17(12), 3065-71.

Van Rooij, I.A., Tonkelaar, I., Broekmans, F.J., Looman, C.W., Scheffer, G.J., de Jong, F.H., Themmen, A.P. &TeVelde, E.R. (2004). Anti-mullerian hormone is a promising predictor for the occurrence of the menopausal transition. Menopause. 11(6 pt 1), 601–6.

Visser, J.A., De Jong, F.H., Laven, J.S. &Themmen, A.P. (2006). Anti-mullerian hormone: a new marker for ovarian function. Reproduction. 131(1), 1-9. PMID: 16388003.

Wang, J., Dicken, C., Lustbader , J.W. &Tortoriello, D.V. (2009). Evidence for a müllerian inhibiting substance autocrine/paracrine system in adult human endometrium. Fertility and Sterility, 91(4), 1195-1203.

Weatherall, D.J. & Clegg, J.B. (2001). Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 79 (8), 704–12. PMID: 1154532.

Weenen, C.,Laven, J.S., Von Bergh, A.R., Cranfield, M., Groome, N.P., Visser, J.A., Kramer, P., Fauser, B.C. &Themmen, A.P. (2004). Anti- mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Molecular Human Reproductive. 10(2), 77-83. doi: 10.1093/molehr/gah015.

Wunder, D.M.,Guibourdenche, J., Birkhäuser, M.H. &Bersinger, N.A. (2008). Anti-Müllerian hormone and inhibin B as predictors of pregnancy after treatment by in vitro fertilization/intracytoplasmic sperm injection. Fertility and Sterility. 90(6), 2203-10.

Yium, J.,Gabow, P., Johnson, A., Kimberling, W. & Martinez, M.M. (1994). Autosomal dominant polycystic kidney disease in black: clinical course and effects of sickle cell hemoglobin. Journal of the American Society of Nephrology. 4(9), 1670-74.

Appendices

Sudan university of sciences and technology

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Serum level of AMH among Sudanese females with sickle cell anemia

Questionnaire

Name:…….

Age:…….

History and duration of diseases:

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Type and duration of treatment:

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