Sudan University of Sciences and Technology

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Assessment of Microalbuminuria and Lactate Dehydrogenase as Early Indicators of Renal Impairment among Sudanese Children with Sickle Cell Anemia in North Kordofan State

تقيم مستوي MAU وإنزيمLDHكعلامات مبكره لمشاكل الكلي لدي الأطفال السودانيين المصابين بالأنيميا المنجلية بولاية شمال كردفان

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الآية

قال تعالي:

قَالَ يَا قَوْمِ أَرَأَيْتُمْ إِنْ كُنْتُ عَلَىٰ بَيِّنَةٍ مِنْ رَبِّي وَرَزَقَنِي مِنْهُ رِزْقًا حَسَنًا ۚ وَمَا أُرِيدُ أَنْ أُخَالِفَكُمْ إِلَىٰ مَا أَنْهَاكُمْ عَنْهُ ۚ إِنْ أُرِيدُ إِلَّا الْإِصْلَاحَ مَا اسْتَطَعْتُ ۚ وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ ۚ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ ﴿٨٨﴾

صدق الله العظيم

سورة هود الآية 88

Dedications

To Soul of my father

To my precious mother

To my brother and sisters

To my beloved husband

To my teachers

To any person who helped me to fullfil this research

Acknowledgments

Abstract

Sickle cell anemia is widely spread throughout the world and the effects of it on human health are serious.

This is a case control study was done in Elobied city in Sudan sickle cell anemia center (SSCAC) during July to September 2016 among Sudanese children with sickle cell anemia.

Hundred Sudanese children were included in this study (60 children with sickle cell disease were selected as test group and 40 healthy children without sickle cell disease as control group (age was matched)) , blood and urine specimen were collected from both groups and microalbuminuria and lactate dehydrogenase were analyzed by using BTS-350 Biosystem spectrophotomer.

Statistical analysis was done by using SPSS computer program , the results showed a significant increase in mean of microalbuminuria (20.15±17.93mg/l) and lactate dehydrogenase (932.86±324.04u/l) in children with sickle cell disease when compared to children without sickle cell disease (control group) P.value=(0.000), (0.000) respectively .

The result showed significant positive correlation between lactate dehydrogenase and microalbuminuria (r=0.533 p.value=0.000).

The results also showed a insignificant correlation between age and microalbuminuria level and a significant positive correlation between age and lactate dehydrogenase level , microalbuminuria (r=0.248 p.value=0.056) and lactate dehydrogenase level (r=0.274 p.value=0.03).

Statistical analysis also showed a significant negative correlation between hemoglobin and microalbuminuia and a significant negative correlation between hemoglobin and lactate dehydrogenase (r=-0.342 p.value 0.008)

and   lactate   dehydrodenase   (r=-0.460   p.value-0.000)   respectively.

The study result revealed that there was significant elevation of lactate dehydrogenase with microalbuminuria in children with sickle cell anemia and sickle cell anemia disease leads to a significant elevation of lactate dehydrogenase with age and significant elevation of MAU and LDH were inversely with Hemoglobin level .

المستخلص

مرض الانيميا المنجليه ينتشر علي نطاق واسع حول العالم وله تاثيرات خطيره علي صحة الانسان .

اجريت هذه الدراسة للمقارنة بين مجموعتي المرضي والاصحاء في الفترة من يوليو الي سبتمبر 2016 تم اختيارهم عشوائيا من مركز السودان للانيميا المجليه بمدينه الابيض ولايه شمال كردفان لتحديد مستوي MAU وانزيم LDH لدي مرضي الانيميا المنجلية .

شملت هذه الدراسة 60 طفل مصاب بمرض الانيميا المنجلية و 40 طفل غير مصاب بالمرض كمجموعة ضابطة تم جمع عينات البول والدم من كلا المجموعتين وتم قياس تركيز MAU وانزيم LDH باستخدام جهاز BTS-350 Biosystem .

اجري التحليل الاحصائي باستخدام SPSS اظهرت النتائج ان هناك زيادة ذات دلالة احصائية في متوسط تركيز MUA (المتوسط=20.15±17.93mg/l) وLDH (المتوسط= u/l 932.86±324.04) لدي الاطفال المصابون بمرض الانيميا المنجلية مقارنة مع المجموعة الضابطة القيمة المعنوية 0.000و0.000 علي التوالي .

اظهرت الدراسات وجود علاقه اجابيه ذات دلاله احصائيه بين مستوي LDH و MAU لدي الاطفال المصابون بمرض الانيميا المنجليه (معامل بيرسون للارتباط=0.533 ومستوي المعنويه= 0.000)

أيضا اظهرت النتائج عدم وجود علاقة بين العمر وMAU (معامل بيرسون للارتباط = 0.248و مستوي المعنويه =0.056), وعلاقة ايجابية بين العمر وانزيم LDH (معامل بيرسون للارتباط = 0.274 و مستوي المعنويه =0.0034).

واظهر التحليل وجود علاقة سلبية ذات دلالة احصائية بين مستوي الهيموقلوبين وMAU (معامل بيرسون للارتباط =-0.0342 و مستوي المعنويه =0.008) وان هناك علاقه سلبيه زات دلاله احصائيه بين الهيموقلوبين وانزيم LDH (معامل بيرسون للارتباط =-0.0460مستوي المعنويه =0.000).

اظهرت نتائج الدراسه أن مرض الانيميا المنجلية يسبب ارتفاع في مستوي MAUو انزيم LDH مقارنة بالمجموعة الضابطة وايضا وجود زياده في MAU مع الزياده في انزيم LDH لدي الاطفال المصابون بمرض الانيميا المنجليه والزيادة في مستوي تركيز انزيم LDH يتناسب تناسب طردي مع العمر والزياده في مستوي MAU وانزيم LDA تتناسب تناسب عكسي مع الهيموقلوبين .

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List of abbreviations

ADP	Adenosine di phosphate
ATP	Adenosine triple phosphate
DM	Diabetes mellitus
DVT	Deep Vein Thrombosis
ESRD	End Stage of Renal Disease
G6PD	Glucose 6 Phosphate Dehydrogenase
GFR	Glomerular filtration rate
HB	Hemoglobin
HbF	Hemoglobin F
HbS	Hemoglobin S
HbSS	Hemoglobin SS
IU	International Unit
LDH	Lactate dehydrogenase
MAU	Microalbuminuria
mRNA	Messenger ribonucleic acid
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotie dehydrogenase
NO	Nitric oxide
RBCs	Red Blood Cells
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SD	Standard deviation
U/L	Unit per Liter
VOC	Vaso –occlusion Crisi

Chapter one

Introduction and literature review

1.Introduction and literature review

1.1. Introduction

The symptoms of anemia are often mild in relation to the severity of the anemia because Hemoglobin S give up oxygen to the tissue relatively easily compared with Hb A . the clinical expression of Hb ss is very variable some patient having all most normal life , free of crises but other develop crises even as infant and may die in early childhood or as young adult. (Hoffbran *et al* , 2006)

This disorder usually presents early in childhood and affects millions throughout the world. It occurs more commonly in people (or their descendants) from parts of tropical and sub-tropical regions where malaria is or was common. One-third of all aboriginal inhabitants of Sub-Saharan Africa, Spanish-speaking regions (South America, Cuba, Central America), Saudi Arabia, India, and Mediterranean countries such as Turkey, Greece, and Italy, carry the gene. Those with only one of the two alleles of the sickle cell disorder are more resistant to malaria, since the infestation of the malaria plasmodium is halted by the sickling of the cells which it infests. In the US, it affects around 72,000 people, most of whose ancestors come from Africa. (Benjamin *et al* , 2007).

Among the challenges in the management of renal complications of Sickle cell disease are identifying early indicators, reducing glomerular damage and progression to end stage renal failure. The relatively higher glomerular filtration rate (GFR) of children with SCD together with tubular secretion of creatinine result in low serum creatinine levels especially in sickle cell anemia patients. Thus biochemical indices like creatinine, urea and electrolytes have been essentially within the normal range in SCD children with renal complications and imminent renal failure, while proteinuria has been identified to be persistent and to increase progressively with severity of renal damage. (Osei et al , 2011)

1.2. Literature review

1.2.1. Sickle cell anemia

In Africa, at least 240,000 children are born each year with sickle cell disease. Historically, in the absence of newborn screening and appropriate treatment, most such children died undiagnosed in early childhood. However, with increasing awareness of the condition and economic and epidemiologic transition, increasing numbers are surviving. Greater investments in basic and applied research in the African context, and increased sensitization or African ministries of health regarding the importance of this condition, could make a substantial difference to the lives and livelihoods of millions of people living with sickle cell disease on the continent and their families.(Thomas *et al*,2016).Sickle cell disease is a group of hemoglobin disorder in which the sickle B-globulin gene is inherited homozygous sickle cell anemia (Hbss) is the most common while the doubly heterozygote condition of HbSc and HbSb that also cause sickle disease .HbS is insoluble and form crystal when exposed to low oxygen tension .Deoxygenated sickle hemoglobin polymerized in to long fiber, each consisting of seven inter wined double strand with cross linking .The red cells sickle and may block different area of the microcirculation or large vessel causing infract of various organ .(Hoffbrand *et al* ,2006).

Sickle cell disease is caused only by the Hb S allelic variant of the P-globin gene. All individuals who are homozygous or compound heterozygous for Hb S exhibit some clinical manifestations of sickle cell disease. Symptoms usually appear within the first 6 months of life, but there is considerable variability in the severity of the disorder . (Ashley e*tal*,2000)

Hemoglobin (Hb) sickle cell (SC) disease is the second most frequent hemoglobinopathy after homozygous sickle cell disease, also called sickle cell anemia (SCA). There are an estimated 54,736 babies born with HbSC disease each year worldwide. The primary event in the pathogenesis of SCA is HbS polymerization occurring in deoxygenated erythrocytes. The sickled

erythrocytes obstruct vessels and have a reduced red cell life span, leading to hyperhemolysis, diffuse vasculopathy and to tissue damage in various target organs. Hemoglobin composition In HbSC erythrocytes is approximately 50% HbS and 50% HbC. While individually HbS and HbC trait have no clinical consequence, HbSC is accompanied by significant clinical abnormalities.

(Francois*et al,*2012).

1.2.1.1.Distribution of sickle cell anemia in Sudan

Central Sudan: Sickle cell gene is known to be   prevalent in the Khartoum area, which is the capital of the country and situated in central Sudan. In the 1980s when drought and famine struck western Sudan, a huge number of migrations took place and many tribes settled around Khartoum. This unique situation made Khartoum a multiethnic area, with a blend of almost all the Sudanese tribes. Among 632 patients attending various clinics at the Khartoum Teaching Hospital, there were 5.1% with Hb AS and 0.8% with Hb SS . In the Blue Nile area, where groups of indigenous population live, the prevalence ranges from 0-5% in addition to a rate of 16% among some immigrant tribes from western Sudan and West Africa in the area (Elderdery *el al*, 2011) .

Northern Sudan: Although the data about sickle cells gene in the north of Sudan is incomplete, it seems that this area shows a low frequency of SCA. A study conducted in the north of Sudan in Shagia and Manaseer tribes confirmed that the sickle cell gene is lower in the north of Sudan than in other areas . Shagia are partly nomadic, isolated, and an agricultural population. Therefore, it is difficult to determine significantly whether they are Arab or African. Manaseer tribe is of Arab origin. Both of them inhabit the 4th cataract region (Podhorodecka *et al*, 2012).

Western Sudan: The presence of HbS is already well documented among Kordofan and Darfur region inhabitants, especially Albaggara, an Afro-Arab constellation of tribes with a predominantly African descent . (Bayoumi *et al* 1985).

Some findings of a study conducted in Elobied hospital in north Kordofan state, showed that sickle cell trait in relatives of patients suffering from sickle cell disease (SCD) who were referred to this Hospital, was 54% of target samples, which concentrated mainly in two tribes, Bederia and Fulani. Sickle cell disease in Messeryia of Darfur and Messeryia Hummer of Kordofan showed a prevalence of 30.4% and 18% respectively. It is estimated that one in every 123 children born in Messeryia tribe is at risk of having SCD. (Elderdery *et al* 2011). 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. (Attalla *et al*, 2006).

1.2.1.2. Complication of sickle cell anemia

 The protean clinical features of SCD result from chronic variable intravascular hemolytic, micro vascular ischemia and organ damage. Vaso-occlusion is the outcome of a dynamic combination of abnormalities in hemoglobin structure and function, red blood cell membrane integrity, erythrocyte density, endothelial activation, micro vascular tone, inflammatory mediators, and coagulation. These path physiologic events translate into clinical manifestations that fall into four general categories: anemia and its squeal; vaso-occlusive crises and bone marrow fat embolization syndrome; infection (from functional asplenia) and organ dysfunction. Organ damage results from a combination of hemolytic and infarction and may be manifested as stroke, retinopathy, nephropathy, liver disease or pulmonary arterial hypertension. Intravascular hemolytic in SCD causes the release of hemoglobin into the plasma. When the capacity of protective hemoglobin-scavenging mechanisms (haptoglobin and hemopexin) has been saturated, levels of cell-free hemoglobin increase in the plasma resulting in the consumption of nitric oxide (NO) by hemoglobin-mediated NO scavenging. In addition, arginase released by hemolyzed red cells can deplete blood plasma of arginine, the substrate for NO production by NO synthase. NO plays a major role in vascular homeostasis and is a critical regulator of smooth muscle relaxation and vasomotor tone, expression of endothelial adhesion

molecules and platelet activation and aggregation. A deficiency in NO, due to its inactivation by cell-free plasma hemoglobin levels during intravascular hemolytic in SCD, may underlie complications associated with SCD. (Zakari *et al*, 2008)

Fever, dehydration, hypoxia, acidosis, stress, and a cold environment may precipitate sickling, although a precursor event is not always identified. The path physiology of SCD is considerably complex, involving abnormalities of hemoglobin, the RBC's membrane, erythrocyte hydration, the endothelium, vascular tone, inflammatory responses, leukocytes, and coagulation. This forceful combination of factors results in cell interactions, generating hemolytic and micro-vascular obstruction, ultimately leading to damage of nearly all organ systems.(Uche *et al*, 2008).

1.2.1.3. Effect of sickle cell disease on the kidney

Among the challenges in the management of renal complications of SCD are identifying early indicators, reducing glomerular damage and progression to end stage renal failure. The relatively higher glomerular filtration rate (GFR) of children with SCD together with tubular secretion of creatinine result in low serum creatinine levels especially in sickle cell anemia patients. Thus biochemical indices like creatinine, urea and electrolytes have been essentially within the normal range in SCD children with renal complications and imminent renal failure, while proteinuria has been identified to be persistent and to increase progressively with severity of renal damage. (Osei and Rodrigues 2011)

Nephropathy is a serious complication of SCD that begins in childhood and may progress to overt renal failure. Sickle cell nephropathy involves damage to multiple structures within the kidney, including the glomeruli within the renal cortex and the renal tubules and vasa recta within the hypoxic, hyperosmolar renal medulla. ESRD develops in 4.2 to 11.6% of adults with HbSS and is an independent predictor of premature mortality in young adults . Common clinical markers of renal function such as serum creatinine are not reliable indicators of early stage glomerulopathy in SCD because of the increased

GFR, lower muscle mass, and increased tubular secretion of creatinine in individuals with SCD . (Marianne *et al ,* 2011).

Glomerular changes begin as early as the first decade of life in otherwise asymptomatic SCD patients . Early glomerular changes in SCD are characterized by high renal blood flow, glomerular hyperfiltration and hypertrophy and a gradual loss of glomerular filtration perm selectivity such that larger molecules such as albumin abnormally permeate the restrictive pores of the glomerular capillary wall. Thus, albuminuria is a sensitive and early clinical marker of glomerulopathy . (Marianne *et al ,* 2011).

1.2.1.4. Treatment 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 penicillinshould start at diagnosis and continue at until puberty. Hepatitis B vaccinationis also given as transfusions may be needed. Crises- treat by rest, warmth, rehydrationby oral fluids and intravenous normal saline and antibiotics if infection is present. Analgesiaat the appropriate level should be given; suitable drugs are paracetamol, a non-steroidal anti-inflammatory agent and opiates, e.g. continuous subcutaneous diamorphine. Blood transfusionis 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 all immunization 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 hemolytic, 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).

Stem cell transplantationcan cure the disease and many patients have now been successfully treated. The mortality rate is less than 10%. Transplantation is only indicated in the severest of cases whose quality of life or life expectancy 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).

1.2.2. Microalbuminuria

The urinary protein called albumin is increasingly recognized as the earliest sign of vascular damage in the kidney. The phenomenon of albuminuria has been recognized for more than 200 years, and its association with kidney disease dates to the epochal insights of Richard Bright in 1827. (Glassock , 2006)

is defined as persistent albuminuria in the range of 30 to 299 mg/24 h or an albumin-creatinine ratioof 30 to 300 g/mg. Clinical proteinuria or macroalbuminuria is established with an albumin-creatinine ratioof _300 mg/24 h or an albumin-creatinine ratio of_300 g/mg . (Michael , 2003).
Microalbuminuria is caused by glomerular capillary injury and so may be a marker for diffuse endothelial dysfunction . According to Steno hypothesis, albuminuria might reflect a general vascular dysfunction and leakage of albumin and other plasma macromolecules such as low density lipoproteins into the vessel wall that may lead to inflammatory responses an in turn start the atherosclerotic process . (Wrone *et al* , 2003).

1.2.2.1. Clinical significant of microalbuminuria

High blood pressure may cause microalbuminuria by increasing glomerular filtration pressure and subsequent renal damage. It is possible that the development of microalbuminuria is a marker for pathophysiologic events that aggravate blood pressure or impair the response to the BP-lowering effects of antihypertensive drugs or, alternatively, that the increasing systemic arterial BP transmits a higher pressure to the glomerular and per tubular capillaries (in the presence of afferent arteriolar dilation), thereby promoting abnormal glomerular perms electivity or changes in tubular albumin processing (Glassock , 2006)

Even high normal blood pressure is associated with significant higher frequency of microalbuminuria and this way may be a biomarker of increased cardiovascular risk. There may be also common genetic factors that predispose to both high BP and microalbuminuria(Knigh *et al* , 2003).

Albuminuria is often associated with metabolic syndrome, a syndrome of insulin resistance, obesity, hypertension, dislipidemia, and increased renal and cardiovascular morbidity. Several evidence suggest that insulin resistance precedes and probably contributes to the development of microalbuminuria in diabetic patients as well as in non diabetic subjects.(Mykkanen *et al* , 1998).

It has been shown that subjects with microalbuminuria are more insulin resistant than those with a normal urinary albumin excretion, and that the magnitude of insulin resistance is independently associated with microalbuminuria. Thus increased albuminuria could be taken as an indicator of insulin resistance and of the increased renal and cardiovascular risk associated with the metabolic syndrome.(Ruggenenti . Remuzzi , 2006).

Diabetes mellitus causes progressive changes to the kidneys and ultimately results in diabetic renal nephropathy. This complication progresses over years and may be delayed by aggressive glycemic control. An early sign that nephropathy is occurring is an increase in urinary albumin. Microalbumin measurements are useful to assist in diagnosis at an early stage and before the development of proteinuria. An annual assessment of kidney function by the determination of urinary albumin excretion is recommended for diabetic patients (Michael , 2003)

1.2.3. Lactate dehydrogenase

Lactate dehydrogenase (LDH) is one of the enzymes of the glycolytic pathway that catalyzes the conversion of pyruvate to lactate with concurrent conversion of NADH to NAD$^+$. It is a ubiquitous enzyme found in all tissues. Serum LDH exists in 5 separable isoenzymes numbered 1-5 according to their electrophoretic mobility The distribution of the 5 isoenzymes is not uniform across body tissues. LDH1 and LDH2 are found primarily in RBCs and heart muscle; LDH3 is highest in the lungs; LDH4 is highest in the kidneys, placenta, and pancreas; and LD H5 is highest in skeletal muscle and liver . (Samir 2013).

Lactate dehydrogenase (LD) is an enzyme in the glycolytic pathway and is released as a result of cell damage. (Teitz . 2005)

1.2.3.1. Lactate dehydrodenase regulation

LDH in humans uses His(193) as the proton acceptor, and works in unison with the coenzyme (Arg99 and Asn138), and substrate (Arg106; Arg169; Thr248) binding residues. (Holmes RS and Goldberg 2009). The His(193) active site, is not only found in the human form of LDH, but is found in many different animals, showing the convergent evolution of LDH. The two different subunits of LDH: LDHA also known as the M subunit of LDH, and LDHB also known as the H subunit of LDH both retain the same active site, and the same amino acids participating in the reaction. The noticeable difference between the two subunits that make up LDH's tertiary structure is the replacement of alanine (in the M chain) with a glutamine (in the H chain). This tiny but notable change is believed to be the reason the H subunit can bind faster, and the M subunit's catalytic activity isn't reduced when subjected to the same conditions as the H subunit; while the H subunits activity is reduced fivefold . (Eventoff*et al* ,1977) .

LDH is also regulated by the relative concentrations of its substrates. LDH becomes more active under periods of extreme muscular output due to an increase in substrates for the LDH reaction. When skeletal muscles are pushed to produce high levels of power, the demand for ATP in regards to aerobic ATP supply leads to an accumulation of free ADP, AMP, and Pi. The subsequent glycolytic flux, specifically production of NADH and pyruvate, exceeds the capacity for pyruvate dehydrogenase and other shuttle enzymes to metabolize pyruvate. The flux through LDH increases in response to increased levels of pyruvate and NADH to metabolize pyruvate into lactate . (Spriet *et al* , 2000)

LDH undergoes transcriptional regulation by PGC-1α. PGC-1α regulates LDH by decreasing LDH A mRNA transcription and the enzymatic activity of pyruvate to lactate conversion. (Summermatter *et al*, 2013).

1.2.3.2. Biological role of lactate dehydrogenase

LDH catalyzes the interconversion of lactic and pyruvic acids using the coenzyme NAD . The reaction can proceed in either a forward (lactate[L]) or reverse (pyruvate [P]) direction. Both reactions have been used in clinical assays. The rate of the reverse reaction is approximately three times faster, allowing smaller sample volumes and shorter reaction times. However, the reverse reaction is more susceptible to substrate

Exhaustion and loss of linearity. The optimal pH for the forward reaction is 8.3 to 8.9; for the reverse reaction it is 7.1 to 7.4. (Michael et al , 2005)

1.2.3.3. Clinical significant of lactate dehydrogenase

LDH has long been considered a useful clinical marker of intravascular hemolysis. Its serum levels are mildly elevated in extra vascular hemolysis, such as immune hemolytic anemia, but are substantially elevated with intravascular hemolysis, such as thrombotic thrombocytopenic purpura and paroxysmal nocturnal hemoglobinuria. Although in sickle cell disease two thirds of hemolysis occurs extravascularly, the remaining one third of red cells hemolyze intravascular, potentially releasing as much as 10 g hemoglobin per day into blood plasma. This robust hemolytic rate increases even further during vasoocclusive pain crisis (VOC). Elegant biochemical studies performed 35 years ago have demonstrated significant increases in serum LDH and plasma hemoglobin levels, the gold standard marker of intravascular hemolysis, during VOC. (Geregory *et al*, 2005)

Because of its widespread activity in numerous body tissue LDH is elevated in a variety of disorders. Increased levels are found in cardiac, hepatic, skeletal muscle, and renal diseases, as well as in several hematologic and neoplastic disorders. The highest levels of total LDH areseen in pernicious anemia and hemolytic disorders.Intramedullary destruction of erythroblasts causes elevationas a result of the high concentration of LDH in erythrocytes. Liver disorders, such as viral hepatitis and cirrhosis. (Michael *et al* , 2005)

LDH is generally high at steady state in sickle cell disease and comes from multiple sources, representing damage to cells from several different organs. It showed at steady state an average of 71% of total LDH was derived from a

combination of LD1 and LD2, reflecting disproportionate elevation of isoenzymes that are consistent with red cell origin .(Gerogory *et al* , 2013)

An elevated serum of lactate dehydrogenase (LDH) was observed in the sickle cell patient population in steady state . The elevation of LDH was associated with hemolysis, pain crisis, pulmonary hypertension, leg ulcer, kidney damage and endothelial activation with elevated soluble vascular adhesion molecule .. The identification of level of LDH may be considered as a marker of hemolysis and might be an important tool for   the early detection of the severity of the disease in SCA individual.(Tite*et al* ,2015)

1.3. Rationale

Sickle cell disease is serious condition affecting the blood and various organs in the body sickle cell anemia is associated with wide spectrum of renal abnormality.

Number of researches indicate that sickle cell disease has health effect on kidney function.

No studies were found in Sudanese children with sickle cell anemia to evaluate the microalbuminuria and lactate de hydrogenase as early indicator of renal impairment.

1.4. Objective

General objective:

To study level of lactate dehydrogenase and microalbuminuria as early indicator of renal impairment among Sudanese children with sickle cell anemia

Specific objective:

1/To measure serum lactate dehydrogenase and microalbuminuria in Sudanese children with sickle cell anemia in comparison to healthy individuals.

2/ To correlate between microalbuminuria and lactated dehydrogenase among children with sickle cell disease.

3/To correlate between level of micraalbuminuria and lactate dehydrogenase with age and hemoglobin.

Chapter two

Material and methods

2. Material and methods

2.1. Materials

2.1.1. Study design

This is a descriptive analytical case control study.

2.1.2. Study area

Elobied city, patients whom attended to Sudan Sickle Cell Anemia Center (SSCAC)

2.1.3. Study population

This study included 60 sickle cell anemic children and 40 healthy individual as control during July to September

2.1.4. Inclusion criteria

Sudanese children with sickle cell anemia

2.1.5. Exclusion criteria

Individual with hypertension, DM, renal disease or any disorder that may affect the level of LDH were excluded.

2.1.6. Ethical consideration

All participants were told about the research importance during interview and all of them were agree to participate. Data was collected by using questionnaire (Appendix I).

2.1.7. Sample size and sampling technique

About 3 ml of venous blood collected from each patient at plain container after clotting, centrifuged for 3 min at 3000 RPM to obtain serum

Fresh urine sample in clean tube, centrifuged at 3000 RPM

2.1.8. Data analysis

Was done by using computer program SPSS version 16.

2.1.9. Quality control

The control material (normal and abnormal) were used in this study and the value obtained fall within the defined limits.

2.2 Methods

2.2.1. Microalbuminuria estimation

Principle of method:

Albumin in the urine sample causes agglutination of the latex coated with anti-human albumin. The agglutination of the particles is proportional to the albumin concentration and can measure by turbidity.

(Appendix II).

2.2.2. LDH estimation

Principle of method:

Lactate dehydrogenase (LD or LDH) catalyzes the reduction of pyrovate by NADH to form lactate and NAD . The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm

Pyruvate + NADH+ H $\longrightarrow$ Lactate + NAD . (Appendix III).

Chapter three

Results

3 . Results

Sixty sickle cell anemic patients were enrolled in this study to assess the effect of sickle cell anemia on lactate dehydrogenase level and microalbuminuria amount. And forty healthy individuals were served as control group

BTS-350 Biosystem spectrophotometer was used for estimation of serum LDH and MAU level.

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

Table.3.1. Shows the general characteristic of patients.

Table.3.2. Shows the mean concentration of (LDH U/L) and (MAU mg/L) level among case and control .

Mean ±SD for case versus control :

(20.15±17.93mg/l versus 9.43±3.29mg/l) for Microalbuminuria

(932.86±324.04u/l versus 460.80±149.05u/l) for Lactate dehydrogenase.

Figure.3.1. a scatter plot shows significant positive correlation between(MAUmg/l) and(LDHu/l) consider as (r=0.533, p.value=0.000)

Figure.3.2. a scatter plot shows insignificant correlation between (MAU mg/l) and age consider as (r=0.248, p.value=0.056).

Figure.3.3. a scatter plot shows a significant negative correlation between (MAU mg/l)and Hb g/dl level consider as (r=-0.342, p.value=0.008)**.**

Figure.3.4 . a scatter plot shows a significant positive correlation between (LDH u/l) and age consider as(r=0.274, p.value=0.034).

Figure.3.5. a scatter plot shows significant negative correlation between (LDH u/l) and Hbg/dl level consider as (r=-0.468, p.value=0.000).

Table.3.1 General Characteristics of study group

| Parameters	Mean±SD (case)	Mean±SD (control)
Age	5.63±3.50	5.80±3.40
Hb	7.52±1.54	12.30±1.51
Gender		
Male	34 (56.7%)	20(50%)
Female	26 (43.3%)	20(50%)

Table 3.2 mean concentration of LDH (u/L) and micro Albumin (mg/L) level among case and control

| Varibale		Mean±SD	P-value
LDH (u/L)	Case	932.86±324.04	0.000
	Control	460.80±149.05	
	Case	20.15±17.93	0.000
Micro Albumin (mg/L)	Control	9.43±3.29	

Independent sample T test was used for comparison , value considered significant at level ≤ 0.05
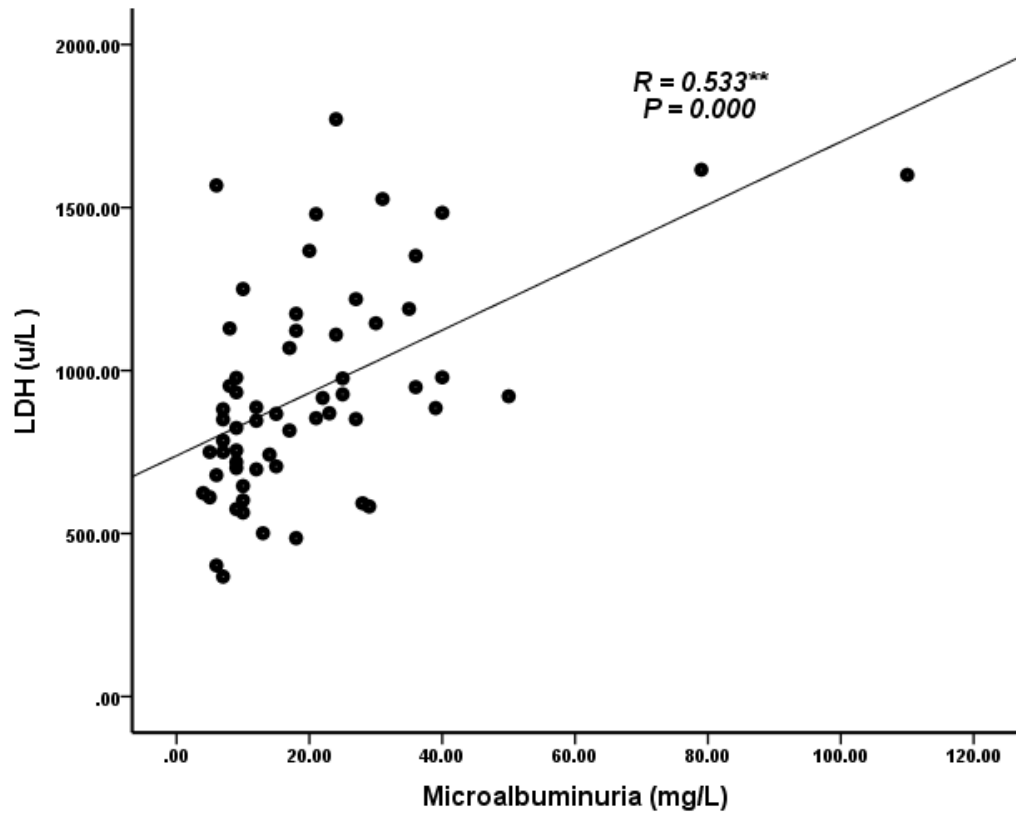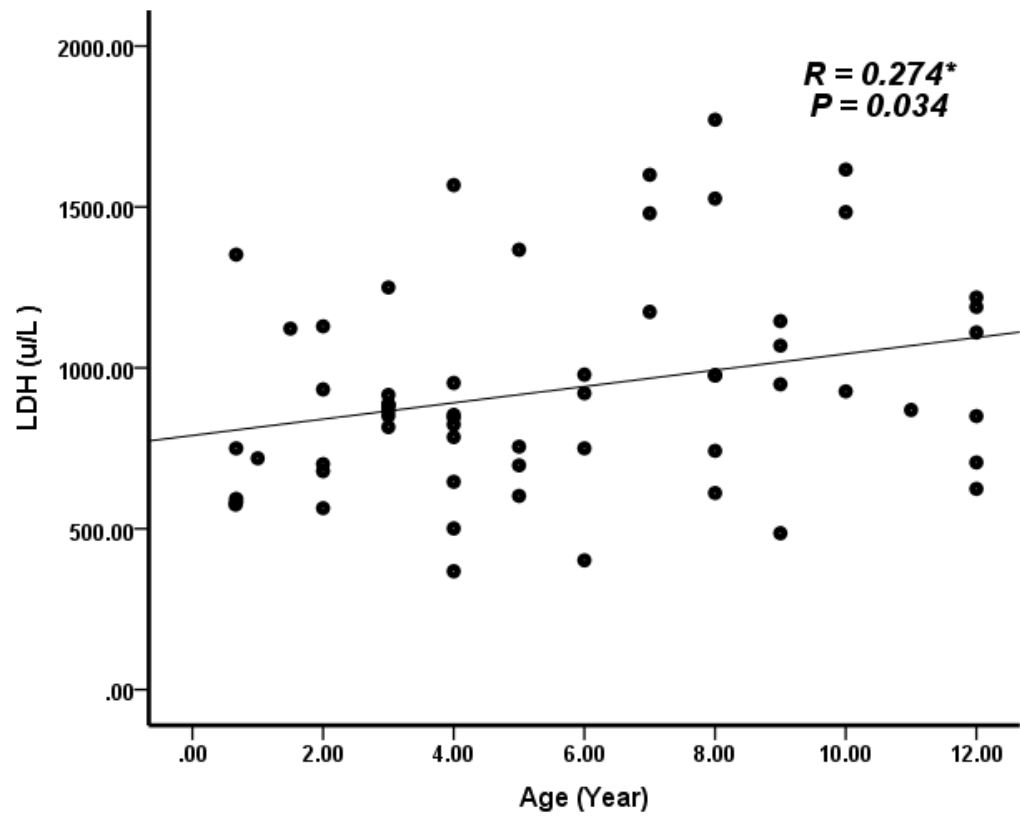
Figure 3-1: correlation between LDH(u/l) and microalbuminuria (mg/l)
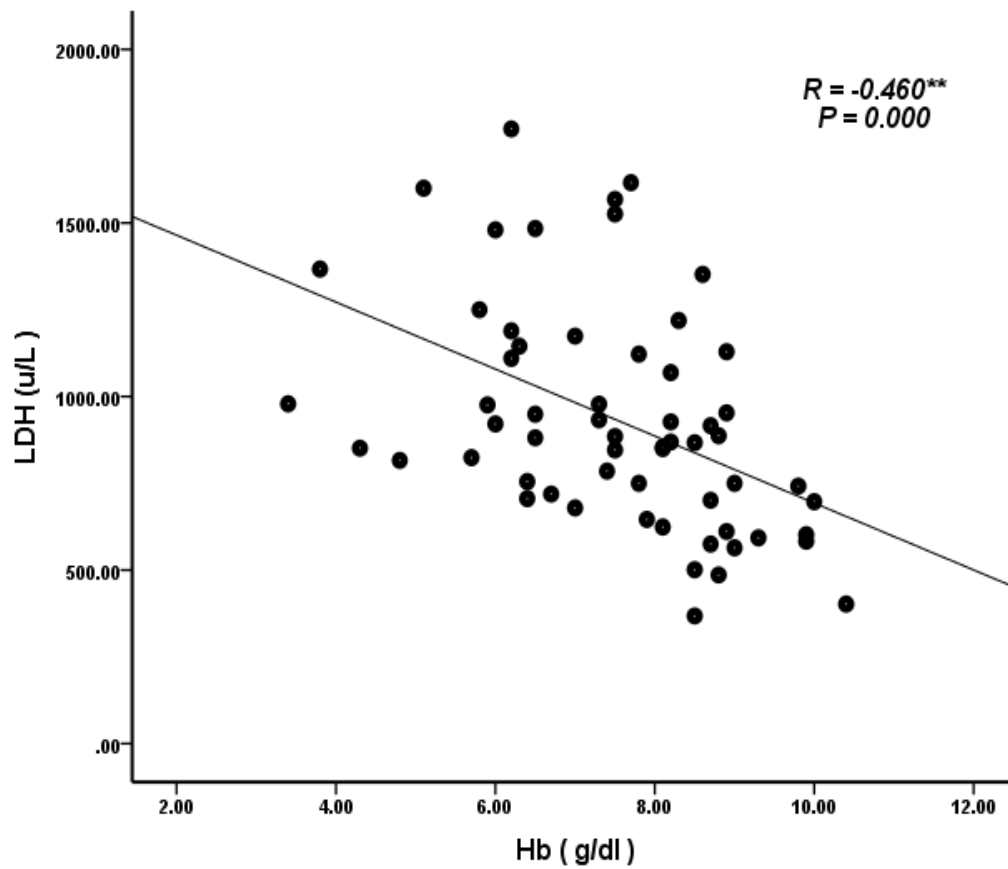significant consider as p-value ≤0.05.
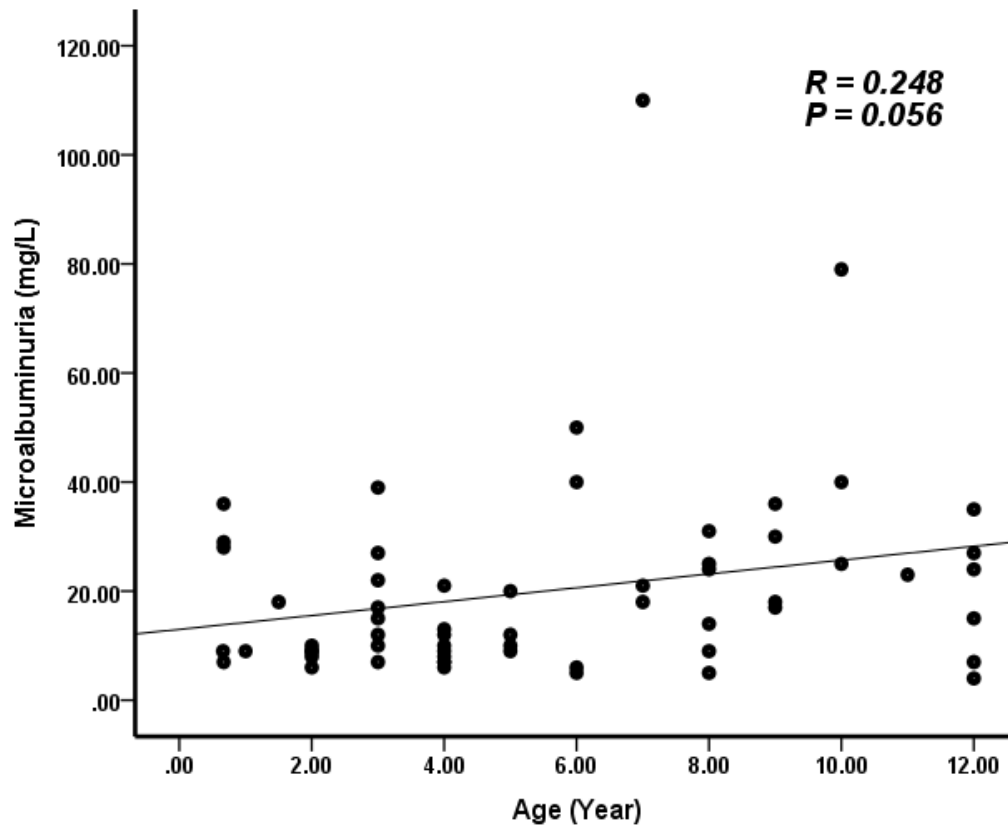
Figure 3-2 : Correlation between microalbuminuria (mg/L) age, significant consider as *p*-value≤0.05.
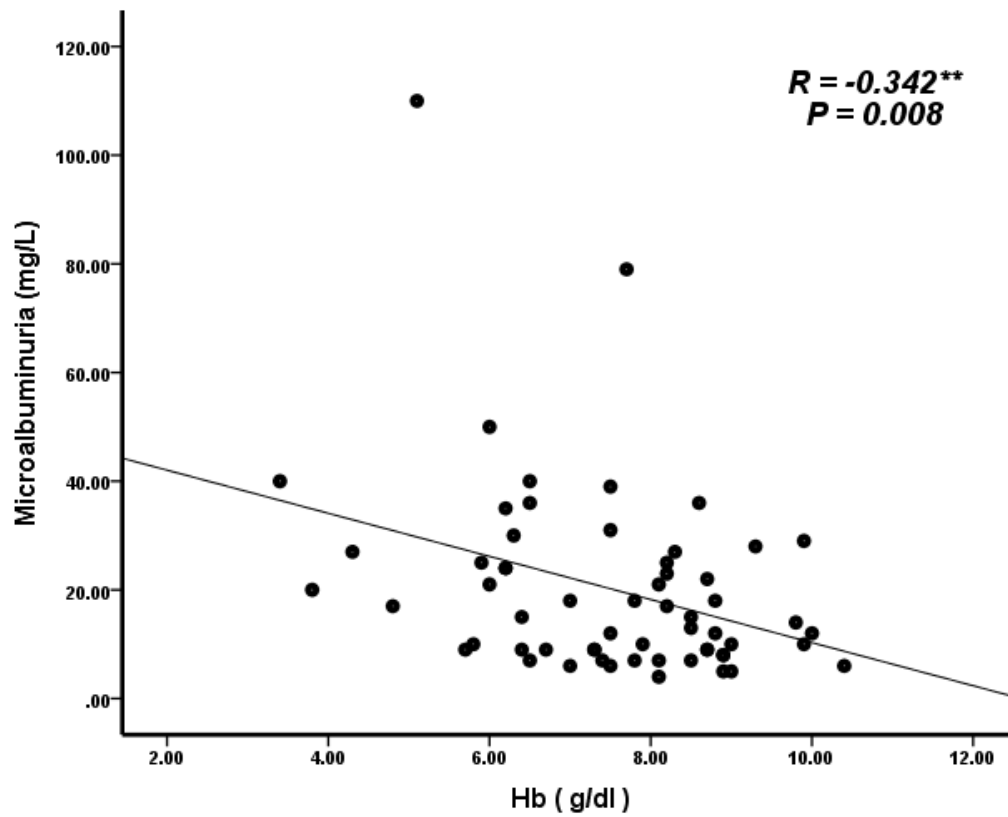
Figure 3-3: Correlation between microalbuminuria (mg/L) and Hb (g/uL),
significant consider as *p*-value ≤0.05.

Figure 3-4 : Correlation between LDH (u/L **)** and age,significant consider as *p*-value ≤0.05.

Figure 3-5: Correlation between LDH (u/L **)** and Hb (g/dL), significant consider as *p*-value ≤0.05.

Chapter four

Discussion, conclusion and recommendation

4 . Discussion , conclusion and recommendation

4.1. Discussion

Chronic kidney disease is one of the leading causes of mortality in patients with sickle cell disease. However, it has been almost exclusively studied in patients with the SS phenotype and in high-income countries, despite more than 80% of patients living in Africa.(Rangue *et al*, 2014).

This is case control study aimed to study the effect of sickle cell anemia on lactate dehydrogenase level and microalbuminurea level. One hundred Sudanese children (60 sickler and 40 non- sickler) , were enrolled in this study. After evaluation of level of lactate dehydrogenase and micro-albuminuria by using spectrophotometer. The statistical analysis was done by using SPSS computer program.

The result showed that significant positive correlation between LDH and microalbuminuria .This agree with study done in Saudi Arabia, they studied the correlation between LDH and other biomarker of kidney function, the analysis shows significant correlation between LDH and creatinine clearance (p.value=0.0008). (Mohammad et al. 2015).

The result showed that lactate dehydrogenase level was significantly higher in sickle cell anemia patients when compared to non- sickle group, and level of micro albuminuria was significantly higher in sickle cell group . This result agree with study done in Sub- sahran Africa by Ranque and his team, Hyper hemolysis is associated with albuminuria(P.Value<0.00001) and high lactate dehydrogenase concentrations(P.value<0.00009).(Ranque,et al. 2014)

Results of this study revealed that there was no corrrlation between microalbuminuria and age, there waspositive correlation of lactate dehydrogenase level and age. But microalbuminuria and lactate dehydrogenase were inversely with hemoglobin concentration.

4.2. Conclusion:

The study results concluded that:

1- The level of lactate dehydrogenase and microalbuminuria were significantly increased in sickle cell disease children compared to non- sickle cell disease children.

2- The level of lactate dehydrogenase was positivity correlate with microalbuminuria among children with sickle cell disease.

3- The level of lactate dehydrogenase was positively correlated with age, no correlation between microalbuminuria and age.

4- The level of lactate dehydrogenase and microalbuminuria were negatively correlated with hemoglobin level in sickle cell disease children.

4.3. Recommendations:

From the finding of this study it recommended that:

Lactate dehydrogenase and microalbuminuria must be carefully evaluated in children with sickle cell anemia to avoid progression of renal problem.

• Further studies should be done to evaluate levels of vitamin D, calcium and phosphate that may be disturbed according to renal insufficiency.

• Measurement of cystatin C recommended as it's widely taken as sensitive markers for the glomerular filtration rate (GFR).

. Study of LDH iso enzyme especially LDH

　　. Estimate Albumin to creatinine ratio .

• 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 renal function tests, micro-albuminuria, LDH, minerals, vitamins and follow up is recommended. Because when renal issues take higher priority in sickle cell disease community; improve survival, reduce morbidity and improve quality of life will definitely occur.

References

References

- **Ashley, K.,** Yang.Q., and Olney,R.S. (1999). Sickle hemoglobin (Hbs) allel and sickle cell disease. Human Genome epidemiology American journal of epidemiology. 151(9):839-845.

  Attalla, B., Mohammed, A.O., Bashir, F.M., Ahmed,F.E., El Hassan, A.M., Ibauf, G., Cavalli,l.l., Karrar,Z.A. and Ibrahim, M.E. (2006). Relationship of the sickle cell gene to the ethnic and geographic groups populating the Sudan. Community Genet .9(2):113–120.

  Bayoumi, R.A., Taha,T.S. and Saha,N.A. (1985). Study of some genetic characteristics of the Fur and Baggara tribes of the Sudan. AmJ Phys Anthropol.67(4):363–370**.**

  Benjami, L.J. and Payne, R. (2007). Pain in sickle cell disease: a multidimensional construct. In: Pace B, editor.Renaissance of Sickle cell disease Research in the Genomic Era. London: Imperial College Press. pp. 99–118.

- **Carl, A.,** Bart,s E.R., Ashood, R., and David, E.B. (2006). Tietz fundamental of clinical chemistry. 6 th : pp 360-361.

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

  Eventoff , W., Rossmann, M.G., Taylor, S.S., Torff, H.J., Meyer ,H., Keil, W., and Kiltz, H.H. (Jul 1977). Structural adaptations of lactate dehydrogenase isozymes. Proceedings of the National Academy of Sciences of the United States of America. 74 (7): 2677–2681.

- **Francous, L.**, Nadjib, H., Katia, S.S., Virgininie, A., Gilles, G., Robert, G. and Jean, P.H,. (2012). Hemoglobin sickle cell disease complication. Hematologica hematol . 97(8)1136-1141.

- **Gergory, J.K.**, Vicki, M.G., Roberto, F.M., Jane, A.J., Taylor, V.I., Claudia, R.M., James, S.N., Xunde, W., Mrjana, P., Sidney, M.M. and Mark T.G. (2005). Lactate dehydrogenase as bio marker of hemolysis-associated nitric oxide resistance ,priapism ,leg ulceration, pulmonary hypertension and death in patient with sickle cell anemia. Blood American society of hematology.107(6):2279-2285.

- **Gerogori, J.K.**, Seyed, M.N. and Mark, T.G. (2013). Lactate dehydrogenases and hemolysis in sickle cell disease. Blood American society of hematology. 122(6): 1091-1092.

- **Glassock, RJ**.(2006). Prevention of Microalbuminuria in Type 2 Diabetes: Millimeters or Milligrams. J Am Soc Nephrol.17 (12):3276–3278.

- **HoffBrand, A.V.,** Moss, p.A,. and Pettit, J.E.(2006). Gentic disorderof hemoglobin. Essential heamatology.5 th : 85-90.

    Holmes, R.S. and Goldberg, E. (2009). <u>Computational analyses of mammalian lactate dehydrogenases: human, mouse, opossum and platypus LDHs</u> . Computational Biology and Chemistry. 33 (5): 379-385.

- **Knight, E.L.**, Kramer, H.M. and Curhan, G.C. (2003). High-Normal Blood Pressure and Microalbuminuria. Am J Kidney Doi ;41(3):588–595.

- **Marianne, M.C.,** Pherson, y**.**, shameem, F.J., Ifeyinwa, O. C., Peter, A.L., Jams, R.E. and Antonio, G. (2011). Chronic kidney and albuminuria in children with sickle cell disease . Clin J Am soc nephrol. 6(11): 2628-2633.

- **Mohammad, S.**A., Shaker, A.M., Abdulkareem, M.A.and Rana, M.H.(2015). Lactate dehydrogenase as biomarker for early renal damage in patients with sickle cell disease. Saudi Journal of kidney Disease and Transplantation.26(6):1161-1168.

- **Mykkanen, L.**, Zaccaro, D.J., Wagenknecht, L.E., Robbins, D.C., Gabriel, M. and Haffiner, S.M. (1998). Microalbuminuria is associated with insulin resistance in nondiabetic subjects: the insulin resistance atherosclerosis study. Diabetes.47 (5):793–800.

- **Osei, C.**T., Yeboah and Rodrigues, O.(2011). Renal ststus of children with sickle cell disease in Accra,Ghana: Ghana Medical journal.45(4):155-160.

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

  Podhorodecka, **A.G**., Knap, O.M., Parczewski, M., Kuleta, A.B. and Ciechanowicz, A. (2011). Sickle Cell Anemia-Associated Beta-Globin Mutation in Shagia and Manasir Tribes from Sudan. Pol J Environ . 20(6):1525–1530.

- **Ranque, B.,** Menet, A.**,** Diop, I.B., Thiam, M.M., Diallo, D., Diop S., Diagne, I., Sanogo ,I., Kingue, S., Chelo,. D, Wamba ,G., Diarra ,M., Anzouan, J.B.,Guetta ,R., Diakite, C.O., Traore ,Y., Legueun, G., Deme,L.y .,Belinga ,S., Boidy, K., Kamara, I., Tharaux, P.L. and Jouven, X.(2014). Early renal damage in patients with sickle cell disease in sub-Saharan Africa. Lancet hematol.1(2):64-73.

- **Ruggenenti, P**. and Remuzzi ,G.(2006). Time to abandon microalbuminuria. Kidney Int.70(7):1214–1222.

- **Samir, K.B**.(2012). Lactate dehydrogenase and hemolysis in sickle cell anemia. Blood Amircan socity of hematology.121(1): 243-244.

  Spriet ,L.L., Howlett, R.A. and Heigenhauser, G.J. (2000). An enzymatic approach to lactate production in human skeletal muscle during exercise. Med Sci Sports Exerc. 32 (4): 756–763.

- **Summermatter, S.**, Santos, G., Pérez-Schindler, J. and Handschin, C. (2013). <u>Skeletal muscle PGC-1α controls whole-body lactate homeostasis through estrogen-related receptor α-dependent activation of LDH B and repression of LDH A.</u> Proc Natl Acad Sci USA .110 (21): 8738–8743.

- **Uche, A.N**, Angie, E.M.and Tochukwu ,I. (2008).pharmacological management of sickle cell disease. PanTpharmacy and therapeutics. 33(4): 238-234.

- **Wrone, E.M.**, Carnethon, M.R., Panaliappan,L.P.and Formann, S.P.(2003). Association of dietary protein intake and microalbuminuria in healthy adults: Third National Health and Nutrition Examination Survey. Am J Kid Dis.41(3):580–587.

- **Zakari, Y.A.**, Ashaunta, R.T. and Gregory, J.K.(2008). Current therapy of sickle cell disease . Hematologica. 91 (1):7-10.

Appendices

Appendix I

Sudan University of Science and Technology

College of graduate studies

Assessment of Microalbuminuria and Lactate Dehydrogenase as Early Iindicators of Renal Impairment Among Sudanese Children with Sickle Cell Anemia in North Kordofan State

Questionnaire

Name:

Age:

Date:

1-Gender:

      Male ( )   Female ( )

3-Family history:

    Yes ( )   No ( )

Hemoglobin level :

Result of LDH:

Result of MAU: