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RESEARCH ARTICLE

FULL BLOOD COUNT IN SUDANESE CARRIERS OF 3.7 ALPHA THALASSEMIA

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ABSTRACT

Introduction: Alpha-thalassemia is a genetic disorders that have high prevalence in the human population around all over the world especially in plasmodium falciparum endemic area, characterised by microcytic and hypochromic anaemia. The carriers for the disease present with a mild anaemia can be missed diagnosed as iron deficiency anemia and the patients could take the iron therapy without response. **Aim:** This a cross sectional hospital base study aimed to find out a guide line to discriminate the high risk patient for alpha thalassemia in Sudan, based on the basic hematological investigation. **Methods:** Based on the FBC of 98 blood samples of highly suspected patient to alpha thalassemia out of 300 patients with undefined microcytosis were selected for Hb electrophoresis, ferritin and PCR. **Results:** Of the 98 patients 7 were carriers for the 3.7 deletion mutation which is the most prevalent mutation in African. In these carriers the RBCs and HCT were significantly increased "*P-value <0.05*". The Hb level revealed mild decrease without statistical significance "*P-value >0.05*". The MCV and MCH were clearly decreased, but the MCHC slightly decreased. The Ferritin level was normal and the RDW_CV clearly increased. The quantitative Hb electrophoresis was normal in addition to the presence of many target cells in peripheral picture and no one of these carriers presence with clinical manifestations indicating for anemia. **Conclusion:** Any patient present with undefined microcytosis, increased RBCs, HCT, RDW_CV with normal Ferritin level, Hb A_2 and target cells in peripheral blood should undergo PCR for the 3.7 Alpha deletion mutation.

INTRODUCTION

The thalassemia is a heterogeneous inheritance disorders caused by mutations that affecting the synthesis of the haemoglobin resulting in the decrease of the alpha or beta globin chains and leading to imbalance of the α: β ratio. (Kumar V. *et al* 2010, Schwarting R. 2008).

The disease was discovered for the first time by Cooley and Lee in 1925, who reported five anemic cases of children presented with hepatosplenomegaly, bone deformity and discoloration of the skin without bile in the urine and their blood showed moderate leukocytosis, nucleated RBCs and many reticulocytes attended Michigan Pediatric Hospital in the USA and they were Mediterranean region origin. (Cooley T.B. 1925) The term "Thalassemia "was used for the description of the disease by Whipple and Bradford in 1932and the word thalassemia is derived from the Greek letter (φαλασσα) which means the sea, but now the disease spread worldwide with high prevalence in tropical and subtropical countries and it characterized by severe anemia associated with splenomegaly and bone deformity. (Weatherall, D. J.2001, Porwit, A. 2011The disease is mostly inherited as Mendelian recessive fashion, and most of the cases can be recognized by simple hematological analysis although the heterozygous cases are normally symptomless, but in case of the homozygous the patient inherit two copies of mutant alpha globin gene with severe clinical manifestations and hematological abnormalities. Annually 300,000 thalassemic patients are born around the world and this highly distribution of the disease is due to the population movements and migration (Weatherall, D. J. 2001). The disease can be classified according to the affected type of globin chain α, β, δβ, γδβ, δ, γ and εγδβ thalassemia. But the important types of the disease are the α and β thalassemia's while the other forms are very rare. In many populations the alpha and beta thalassemia's may associate with a variety of different structural hemoglobin variants because it is commonly to inherit a combination of alpha or beta thalassemia with structural hemoglobin variant genes and the complex interaction will result to different clinical symptoms associated with the thalassemia syndrome (Weatherall, D. J. 2001). The α-thalassemia has a wide range of distribution globally, it affects 5–40% of the population in

Africa and 40–80% in South Asia, and genetically it results from a homologous deletion of an approximately 4 Kb DNA segment that is flanked by two α-globin genes on the pair chromosome 16. (Coleman W.B. 2010)

Globally the distribution of the alpha thalassemia is mainly found in the tropical and subtropical countries as tropical Africa, Middle East, India, Southeast Asia and Pacific Island where the prevalence varies in range between 1-98%, (Vichinsky, E. P. 2000) but the prevalence and frequency of the disease into an increase globally because of the migration worldwide (Liu, Y. T. 2000). In these countries the malaria of plasmodium falciparum is endemic and this have a beneficial importance because it gave a protection against this type of malaria in carrier of the alpha thalassemia, so the patients clinically don't develop severe infection of malaria as observed (Weatherall, D. J. 2001). In the past the diagnosis and detection of such a disease was very difficult but now a days the developing of a new advance molecular biology techniques by DNA analysis make it easy to diagnose cases and enable the scientist to know the different forms of the disease and it's occurrence different races and ethnic group (Clegg, J. B. 1999). Due to the sequestration of the alpha^{0}-thalassemia in Southern China, Thailand, and Vietnam due to intermarriage the prevalence of Hb H and the hydropsfetalis are presence in high account in South East Asian population (Weatherall, D. J. 2001) while the $-\alpha^{3.7}$ deletion mutation is the most prevalent in African population (Old, J. M. 2003, Kattamis, A. C. 1996) where prevalence of this type of mutation was estimated between 20-30% in West Africa (Mockenhaupt, F. P. 1999, Borges, E. 2001).

Objective

The main objective of this study was to search for indicators from the basic hematological parameters and correlate them with the molecular study in order to find out a strategy for the diagnosis of the 3.7 deletion mutation carriers in Sudan.

METHODS

Ethical clearance

The study was certified by ethical research board of Alneelain University at its meeting dated on 15/2/2013 and approved by the ministry of health to collect blood samples from the hospital in Khartoum State. The non probability blood samples collected from the study population after their agreement to participate and provide their data.

Full blood counting (FBC)

2.5 ml of blood were withdrawn from an antecubital vein of each patient (Bain, B. J. 2011) and the Full Blood Counting was done using an automatic multi-parameter blood cell counter for in vitro diagnostic in the clinical laboratories (Helen Diaz 2012).

Quantitative hemoglobin electrophoresis

SAS-MX Alkaline Hb-10 kit (Helena Bioscience Europe) was used for the quantitative haemoglobin electrophoresis. (Helena BioSciences).

Ferritin measurement

Cobas e411 (Roche) full automated system was used for the measurement of ferritin level in the plasma of the patients by the electrochemiluminescence immunoassay (ECLIA) method (Ferritin reagent kit (German). (Roche Cobas e411).

PCR

Gap-PCR was done for the detection of the 3.7 deletion mutation using the Platinum Multiplex PCR Master Mix (Invitrogen Applied Biosystem USA Origin) (Applied Biosystem USA)

The statistical analysis

The SPSS computer software (version 14) used for data analysis and to investigate the significant differences of the resulted hematological counts in compare with the real value by the chi squire test.

RESULTS

The gender distribution in the study population was 60% males and 40% females figure No (1) and the figure No (2) showed the age distribution in the study population. The Hb electrophoresis revealed 85.7% of the study population was normal, where 11.2% were sickle cell trait, 2% shows an increase in Hb A_2 (they might be β-thalassemia trait) and 1% showed an increase in Hb F table No (1). Regarding the 3.7 carrier patients there was no abnormal hemoglobin detected and also the Ferritin level reveal normal level and also no one of these carriers presence with clinical manifestations indicating for anemia. As samarized in table No(2) the RBCs count increased in the carrier males females and children reveal $7.23\pm0.78 \times10^{12}$/L, $7.21\pm0.67\times10^{12}$/L and $5.06\pm0.87\times10^{12}$/L respectively and there is a significant association between the RBCs count and this PCR pattern (*P-value <0.05*).

Table No 1 Hemoglobin electrophoresis result (n = 98)

Type of Hb	All population (n = 98)	
	Number	Percent
AA	84	85.7%
AS	11	11.2%
AA_2	2	2.1%
AF	1	1.0%

Also the HCT shows an increase in the carrier males, females and children reveal 38.70±3.25 L/L, 37.65±2.33 L/L and 35.06±7.38 L/L respectively and the statistic indicate a significant association with this PCR pattern (*P-value <0.05*). The Hb concentration reveal mild decreased the reading were 11.70±0.57 g/dL, 11.25±0.64 g/dL and 11.6±2.95 g/dL in males, females and children respectively, this decrease in the Hb is not statistically significant (*P-value >0.05*). The MCV show microcytosis and reveal 53.60±1.27fl, 52.35±1.63 fl and 69.20±7.49 fl for the males, females and children respectively, also the MCH shows decrease and reveal 16.25±0.92 pg, 15.60±0.57 pg and 22.80±3.44 pg for the males, females and children respectively, while the MCHC slightly decreased and reveal 30.25±1.06 %, 29.90±0.14 % and 32.86±1.64 % for the males, females and children respectively. The RDW_CV in all carriers shows an increase which indicate for the anisopoikilocytosis and it record 20.20±1.70 %, 21.05±0.07 % and 14.86±0.95 % for the males, females and children respectively. The peripheral blood picture the RBCs showed severe microcytosis with anisopoikilocytosis, presence of many target cells and polychromasia.

DISCUSSION

All carriers show no any clinical manifestations of anemia and detected accidentally by a chance through the routine full

blood counting during a regular health check or to investigate the microcytosis and this result agreed with many studies Harteveld C. L. and Higgs D. R. 2010 who reported that most individuals with alpha thalassaemia have a very mild clinical phenotypes which make it not easy be detected during life other than accidently through a routine full blood count is done.

two different combination mutation can affect the RBCs counting in the carriers patients.

The haemoglobin concentration reveals mild anemia it ranged in 11.70±0.57g/dl, 11.25±0.64 g/dl and 11.6±2.95 g/dl in the adult mail, adult female and children respectively and this result agreed with Harteveld and Higgs, 2010 who reported the alpha thalassemia trait patients are characterized by slightly reduced in the haemoglobin level.

Table No 2 Full Blood Count in the 3.7 deletion carriers

Category	RBCs$\times 10^{12}$/L	Hb g/dL	HCT L/L	MCV fL	MCH pg	MCHC %	RDW_CV %
Male	7.23±0.78	11.70±0.57	38.70±3.25	53.60±1.27	16.25±0.92	30.25±1.06	20.20±1.70
Female	7.21±0.67	11.25±0.64	37.65±2.33	52.35±1.63	15.60±0.57	29.90±0.14	21.05±0.07
Children	5.06±0.87	11.6±2.95	35.06±7.38	69.20±7.49	22.80±3.44	32.86±1.64	14.86±0.95

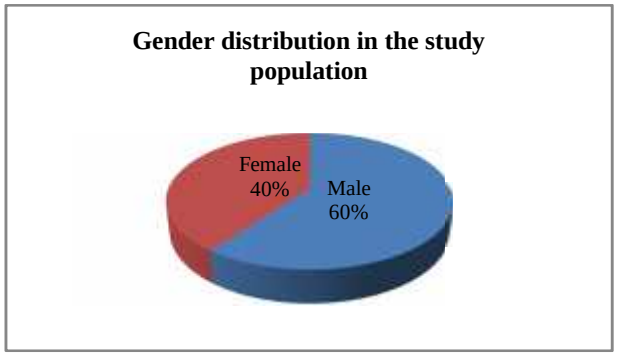

Figure No 1 Gender distribution in the study population

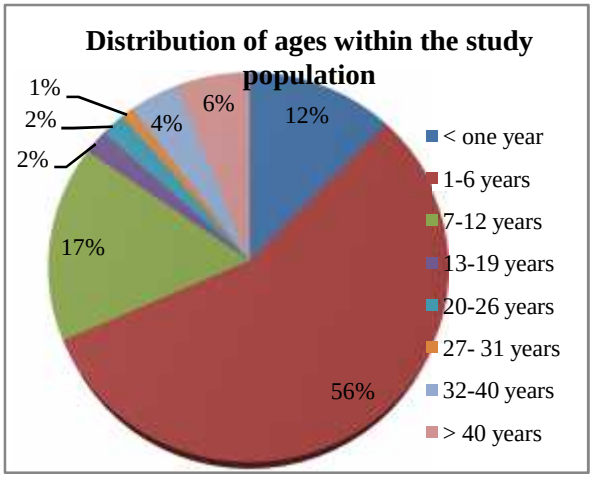

Figure No 2 distribution of the ages within the study population

The RBCs shows a significant increase in their count, so in the adult males ranged in 7.23±0.78 $\times 10^{12}$/L and in the adult female ranged in 7.21±0.67$\times 10^{12}$/L with presence of some target cells (Chi-Square test result in a significant association between the RBCs number and the presence of the mutation "*P-value <0.05*") while in the children they were normal in count ranged in 5.06±0.87$\times 10^{12}$/L, so the increase of the RBCs count is can be consider as a matter of compensation and this finding is agreed with Mwakasungula, S. 2014 & Guimaraes, J. S. 2015 who reported that the RBC was significantly higher than the normal individuals. Also this increase agreed with Akhavan-Niaki H. *et al* 2012 whose their study revealed a compensatory erythrocytosis with the pattern of deletion and point mutation combination of – $\alpha^{3.7}/\alpha^{PA2}\alpha$ the RBCs were 6.28±0.26$\times 10^{12}$/L, so this is also indicate the coinheritance of

The - α3.7 homozygous shows anemia and need blood transfusion. Chi-Square test result in no significant association between the Hb level and the mutation (*P-value >0.05*)

The MCV and MCH showed microcytosis and hypochromasia, in the adult males they ranged in 53.60±1.27fl MCV / 16.25±0.92pg MCH and 52.35±1.63fl MCV / 15.60±0.57pg MCH in the adult female and this result of microcytosis disagree with Harteveld and Higgs, 2010 who reported that the alpha thalassemia trait is characterized by slight microcytosis and hypochromasia and also the degree of the hypochromasia disagreed with Higgs *et al*. 1989 who reported the MCH with the - α3.7 heterozygous is normal and it can be used for the differentiation it from homozygotes for deletional types of α^{+} thalassaemia, or heterozygotes for α^{o} thalassaemia which is usually less than 26pg and always below 27pg, but this microcytosis and hypochromasia result is agreed with Borges, E.*et al* 2001 who conduct a study in the black and Caucasian Brazilian to determine the contribution of the alpha thalassemia to microcytosis and hypochromia, they found 339 adult outpatients with normal hemoglobin (Hb) levels and reduced MCV and MCH. The MCV and MCH are mild decrease in the affected children ranged in 69.20±7.49fl MCV and 22.80±3.44pg MCH. Sankar, V. H. *et al* in 2006 reported a microcytosis and hypochromasis phenotype in 3.7 alpha carrier patients in north India and also Rahim, 2009; Bezerra and Meissner, 2010; Wagner *et al*., 2010 reported the same result and the very interest that reported by Sharma, M. *et al* 2015 who conduct study in patients front ne North of India and his result reveal a median of 68.9fl in 29.6% of the subjects - α3.7deletion heterozygous. Also a hematologic features of alpha thalassemia carriers study done in Iranian by Akhavan-Niaki H. *et al* 2012 revealed a microcytic and hypochromic with patient with coinheritance of missense mutation of the termination codon of HB α2 as Hb Constant Spring (Hb CS) with the – $\alpha^{3.7}$ carrier(- $\alpha^{3.7}/\alpha^{CSP}\alpha$) and also the reported by Azma, R. Z. 2012.This indicate the MCV and MCH in the carrier might be with variation according to the different ethnic groups or might be associated with asymptomatic disease did not discovered at the time of blood sample collection, also may be in this study because the criteria of sample collection depend on the undefined microcytosis and hypochromasis and the small sample size and if the study done on a large population without concentration on the microcytosis and hypochromasis the result might be differ from this, so alpha globin sequencing study and the rest of the other common deletion ($--^{SEA}$, $/--^{MED}$,$/--(\alpha)^{20.5}$, $--^{SEA}$ and $/--^{FIL}$) is very important for these patient because might be there is a combination with one of these other deletion, a combination

with any type of nondeletion defect or presence of secondary mutations as the AC deletion in the vicinity of the initiation codon of the – $\alpha^{3.7}$ allele which reported by Viprakasit, V., *et al* 2003 when they studied two Italian children carrier for the – $\alpha^{3.7}$ deletion with severe microcytosis, hypochromasis and normal iron status. In the homozygous mutation patient the MCV and MCH shows normal with presence of polychromasia, some target cells and anisopoikilocytosis and this may be due to the effect of transfusion.

RDW_CV reveal anisopoikilocytosis, it ranged in 20.20±1.70% and 21.05±0.07% for the adult males and females respectively in compare with the normal populations, while in children shows mild anisopoikilocytosis and their RDW_CV ranged in 14.86±0.95%. This finding is agreed with Vayá, A. *et al* 2011 who evaluated erythrocyte deformability and reported that the Alpha-thalassaemia carriers presented higher red blood cell counts, RDW-CV, lower haemoglobin, MCV, MCH and MCHC than the normal individuals and the same result was reported by Ahmad, R. 2013 and Sharma, M. 2015.

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