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Prevalence of α-Thalassemia 3.7 and 4.2 kb Deletion in Microcytic Hypochromic Anemia Patients from Gaza Strip –Palestine

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Abstract

α-Thalassemia results from impaired α-globin chain synthesis that usually comes about through deletion of α-globin genes. This study was conducted to detect and investigate the prevalence of $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletion mutations in a cohort of microcytic hypochromic anemic patients from Gaza Strip-Palestine. 200 unrelated adult patients, 18 to 48 years old, were recruited from the Hematological departments of the three major Gaza strip hospitals (Al-Shifa, Gaza-European and Nasser). The study participants proved negative upon β-thalassemia carrier screening. Serum iron and total iron binding capacity (TIBC) were tested to exclude iron deficiency. Multiplex-PCR was used for the molecular detection of $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletion mutations. Thirty-one (15.5%) of the investigated cases have α-thalassemia, of which 27 (13.5%) harbored a heterozygous genotype ($-\alpha^{3.7}/\alpha\alpha$) and three (1.5%) wee homozygotes ($-\alpha^{3.7}/-\alpha^{3.7}$). $-\alpha^{4.2}$ deletion was evident in one (0.5%) case only and in a heterozygous state. The frequency of $-\alpha^{3.7}$ and $-\alpha^{4.2}$ alleles were 8.25% and 0.25% respectively. Comparison of hematological parameters between microcytic hypochromic patients with normal genotype and those harboring mutations showed that the $-\alpha^{3.7}/-\alpha^{3.7}$ subjects have lower Hb level and statistically significant difference in the MCV (p =0.006) and MCH (p =0.007). Further studies should be done on normocytic normochromic individuals, since α-thalassemia silent and trait cases may have normal red blood cells indices.

Keywords: α-thalassemia, $-\alpha^{3.7}$, $-\alpha^{4.2}$ deletion mutations, Multiplex-PCR, Gaza Strip

1. Introduction

α-Thalassemia, is a type of hemoglobin disorder that results from reduction or complete absence of α-globin gene synthesis (Muncie and Campbell, 2009) leading to excess of beta (β)-globin or (γ)-gamma chains (Harteveld and Higgs 2010; Galanello and Cao, 2011). α-Thalassemia is the most prevalent single-gene disease in the world (Vichinsky et al., 2005; Harteveld; Higgs, 2010). Clinically, α-Thalassemia can be classified into: α^{+}-thalassemia typically caused by the deletion or dysfunction of one of the four normal α- globin genes and α^{0}-thalassemia resulting from deletion or dysfunction of two α- globin genes. Loss of three or all the four two α-globin genes cause HbH disease [--/-α] and Hb Bart hydrops fetalis syndrome [--/--], respectively (Galanello and Cao, 2011). Two different deletions ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) in the α-globin gene on chromosome 16p13.3, originating from homologous recombination between misaligned chromosomes, represent the most common cause of α-thalassemia (Higgs et al., 1989). The 3.7 kb deletion mutation is encountered worldwide, but it is

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most prevalent in some African, Indian, Nepalese, Sardinian and many other Mediterranean populations, Chinese, and other East Asian populations. A reciprocal recombination between Z segments causes the 3.7 kb deletion producing a chromosome with only one functional α-gene (-$\alpha^{3.7}$ or rightward deletion) lead to α-thalassemia and an α-triplication allele without a thalassemic effect. Similarly, a mutual recombination between mispaired X-boxes results in a 4.2 kb deletion (-$\alpha^{4.2}$ or leftward deletion) (Embury et al.,1980; Higgs et al.,1980; Trent et al.,1981 and Higgs et al.,1984). The prevalence or incidence of α-thalassemia in Gaza Stripe-Palestine is largely unknown. The aim of the present work was to determine the prevalence of the α-thalassemia 3.7 kb and 4.2 kb deletions in patients with microcytic hypochromia.

2. Material and Methods

The two hundred study participants were recruited from the Hematology departments in the three major hospitals in Gaza Strip (Al-Shifa, Gaza-European and Nasser Medical complex). All patients had already undergone premarital carrier screening of β-thalassemia and their results came out negative. The cases included had MCV<80fl and/or MCH<27pg. Ten ml of blood were collected in two EDTA tubes: the 1st tube for CBC and the 2nd for molecular analysis. Also, serum Iron and TIBC were determined. Hematological parameters were analyzed by an automated cell counter (XT1800i, Sysmex, Japan). All data were analyzed by SPSS version 20 and Independent Samples t-test was used. for comparison of the hematological parameters. P value < 0.05 was considered statistically significant.

2.1 Ethical Considerations

The study was approved by the Palestinian Ministry of Health and Helsinki ethical committee. In addition, all the subjects involved in the study gave their oral consent to participate in the study.

2.2 Detection of the α thalassemia mutations

Genomic DNA was prepared from peripheral blood using a commercial kit (Promega, USA). The quality and concentration of isolated DNA were determined by using a Nanodrop spectrophotometer (IMPLEN-USA). Multiplex-PCR was employed in a single reaction tube to assess the presence of -$\alpha^{3.7}$and -$\alpha^{4.2}$ deletions following (Chong et al., 2000). Amplification of a large (2.5 kilobase) segment of the *LIS1* gene 3'UTR (the *LIS1* gene at 17p13.3) was included as a control for amplification success. The sequence of primers used, annealing temperature and the PCR amplicons sizes are demonstrated in table1.

Table 1 The sequence of primers used in the Multiplex-PCR

Name	Sequence (5' to 3')	Product size (bp)	Annealing T
LIS1	**LIS1(F)**- GTCGTCACTGGCAGCGTAGATC **LIS1(R)**- GATTCCAGGTTGTAGACGGACTG	2503	61.5°C
- $\alpha^{4.2}$	**4.2 (F)**- GGTTTACCCATGTGGTGCCTC **4.2 (R)**- CCCGTTGGATCTTCTCATTTCCC	1628	
- $\alpha^{3.7}$	**α2/3.7(F)**- CCCCTCGCCAAGTCCACCC **3.7(R)**-AAAGCACTCTAGGGTCCAGCG	2022/2029	
α2 gene	**α2/3.7(F)**- CCCCTCGCCAAGTCCACCC **α2 (R)**-AGACCAGGAAGGGCCGGTG	1800	

Multiplex-PCR was performed using 15 µl Hot start master mix (Bioline, Germany), 2 µl deionized water, 1 µl DNA template and 0.2 µl of each primer (2 pmol) in one micro-tube (0.2 ml) and were thoroughly mixed. A thermal cycler (BECO-Germany) was used for the PCR amplification with an initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 61.5 °C for 4 min and 72 °C for

4min, and terminated by a final elongation at 72 °C for 5 min. The PCR products were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide solution and visualized on a UV transilluminator. The PCR amplicons sizes were estimated by comparing them with DNA molecular size marker (1kb ladder DNA) run on the same gel.

3. Results

In this study, 200 individuals, including 94 (47.0%) females and 106 (53%) males, were included. All the enrolled cases had low MCV (mean=74.9fl ±4.4) and/or MCH (mean=25.16 pg ±1.8). The serum iron and TIBC proved normal for all participants, the mean value: 107±59 µg/dl and 313±90.5 µg/dl, respectively. Among the 200 investigated subjects, 31(15.5%) were confirmed to be carriers for α-thalassemia deletion mutation. Among them, $-\alpha^{3.7}$ allele presented the highest frequency appearing in 30 subjects (i.e., 15% of the cases). Twenty-seven subjects (13.5%) were heterozygous ($-\alpha^{3.7}/\alpha\alpha$) for the deletion and three individuals were homozygotes ($-\alpha^{3.7}/-\alpha^{3.7}$). The $-\alpha^{4.2}$ deletion was detected in one patient only and in a heterozygous ($-\alpha^{4.2}/\alpha\alpha$) state. Thus, study samples were divided into four genotype which were αα/αα (84.5%), $-\alpha^{3.7}/\alpha\alpha$ (13.5%), $-\alpha^{3.7}/-\alpha^{3.7}$ (1.5%), and $-\alpha^{4.2}/\alpha\alpha$ (0.5%). Table 2 demonstrated the frequency of α-globin alleles and genotypes among the study population.

Table 2 α- globin alleles and genotypes frequency in the study population

Gender	Allele Frequency (%)			Genotype (%)				Total (%)
	αα	$-\alpha^{3.7}$	$-\alpha^{4.2}$	αα/αα	$-\alpha^{3.7}/\alpha\alpha$	$-\alpha^{3.7}/-\alpha^{3.7}$	$-\alpha^{4.2}/\alpha\alpha$	
Male	192(48)	20(5)	-	88(44)	16(8)	2(1)	-	106(53)
Female	174(43.5)	13(3.25)	1(0.25)	81(40.5)	11(5.5)	1(0.5)	1(0.5)	94(47)
Total	366(91.5)	33(8.25)	1(0.25)	169(84.5)	27(13.5)	3(1.5)	1(0.5)	200

A comparison of the hematological parameters between the deletion carriers and non-carriers is shown in Table 3. The comparison showed a significant difference in the means of MCV and MCH levels among $-\alpha^{3.7}/-\alpha^{3.7}$ genotype carriers ($p<0.05$). However, the frequency of $-\alpha^{4.2}$ mutation was too small to include in the statistical comparison.

Table 3 Comparison of hematological parameters between subjects with normal genotype and carries of $-\alpha^{3.7}$and $-\alpha^{4.2}$ deletion

Genotype	Hematological Parameters					
	Hb	**RBCs**	**MCV**	**MCH**	**MCHC**	**RDW**
αα/αα (n =169)	12.14±1.4	4.79±0.52	75.1±4.6	25.2±1.8	33.5±1.7	13.8±1.4
$-\alpha^{3.7}/\alpha\alpha$ (n=27)	12.17±1.6	4.84±0.65	74.6±2.9	25.16±1.7	33.8±1.8	13.7±1.07
P-value	0.91	0.64	0.55	0.89	0.41	0.81
$-\alpha^{3.7}/-\alpha^{3.7}$ (n=3)	11.36±2.3	5.09±0.83	67.6±2.2	22.3±1.01	33.07±2.0	14±0.4
P-value	0.34	0.33	**0.006**	**0.007**	0.66	0.86
$-\alpha^{4.2}/\alpha\alpha$ (n=1)	11.4	4.59	73.7	24.8	33.7	13.1

The genotypes of the silent trait group were: $-\alpha^{3.7}/\alpha\alpha$ and $-\alpha^{4.2}/\alpha\alpha$, while the genotype of the trait group was: $-\alpha^{3.7}/-\alpha^{3.7}$. The comparison between α-thalassemia genotypes showed a significant difference in MCV and

MCH (p= 0.000 and 0.008, respectively) (Table 4). The comparison was also performed for deletion carriers and non-carriers, but the analysis did not reveal any significance (data not shown).

Table 4 Hematological comparison of α-thalassemia silent carriers and trait based on its genotypes

Parameter	Genotypes group*				p-value
	Silent carriers (n= 28)		Trait (n= 3)		
	Range	Mean±SD	Range	Mean±SD	
RBCs($x10^{12}$)	4.06-6.76	4.83±0.64	4.49-6.03	5.09±0.83	0.52
Hb (g/dL)	9.6–17.6	12.14±1.58	10.0-14.0	11.36±2.28	0.44
MCV (fL)	66.8-79	74.5±2.8	65-69	67.5±2.2	**0.000**
MCH (pg)	20.2-26.5	25.15±1.1	21.2-23.2	22.3±1.01	**0.008**

4. Discussion

The frequency of α-thalassemia observed in the investigated microcytic hypochromic anemia showed 14% silent carriers and 1.5% α-thalassemia trait. The total prevalence of α-thalassemia carriers in this study are 15.5%. Lower and higher frequencies where reported from various Middle Eastern countries as illustrated in Table-5. The prevalence of α-thalassemia carriers varies from population to another based on the ethnicity and geographic distribution from high rates in: Oman (58.3%) (Hassan et al., 2010), UAE (49%) (Haj Khelil et al., 2010), Saudi Arabians (43.3%) (Ganeshaguru et al., 1987), Kuwait 39.3% (Adekile et al., 2020), to low frequencies in Lebanon, Syria, Libya (Teebi and Farag, 1997) ,and others as clarified in Table 5.

Table 5 The frequency of α-thalassemia carriers in various Arab populations

Population	% α-thalassemia carriers (no. of sample)	Reference
Gaza Strip-Palestine	15.5% (200)	**Present study**
Egypt	9.25 % (410)	Rizk et al., 2005
Jordan	2.26 % (1,020)	Babiker et al., 1999
Lebanon	Less than 1 %	Teebi and Farag, 1997
Syria	<1–5 %	Teebi and Farag, 1997
Algeria	17.7% (102)	Houcher et al., 2013
Libya	<1–5 %	Teebi and Farag, 1997
Tunisia	5.48 % (44,299)	Fattoum, 2006
Morocco	2.2 %	Benkirane Agoumi and Sebar,2003
Bahrain	24.3 % (10,327)	Mohammed et al., 1992
Kuwait	39.3%(400)	Adekile et al., 2020
Oman	58.3 % (87)	Hassan et al., 2010
UAE	49 % (418)	Haj Khelil et al., 2010
Saudi Arabians	43.3 % (840)	Ganeshaguru et al., 1987
Yemen	8.6 % (699)	Al-Nood, 2009

The $-\alpha^{3.7}$ deletion mutation is a multiethnic mutation and prevalent worldwide, but the frequency of $-\alpha^{4.2}$ deletion is lower and has been shown to be predominant in Southeast Asians and Saudi Arabians (Harteveld et al., 2003 and Yap et al., 2013).

The comparison of $-\alpha^{3.7}$ allele frequency in the present study and some countries is shown in Figure-1. The frequency of $-\alpha^{3.7}$ deletion (8.25%) was almost near the half of that found in the study performed by Hamayel et al. (19.18%) in the Jerusalem -Palestine (Hamayel et al., 2013). Our results showed that the frequency $-\alpha^{3.7}$ allele were higher than that reported in Morocco [0.33%] (Laghmich et al., 2019), Algeria [2.9%] (Mesbah-Amroun et al., 2008),Sicily [4.1%] (Zorai et al., 2002), Tunisia [4.5%](Zorai et al., 2002),

Lebanon [6.08%] (Farra et al.,2015), Sudan [7.2%] (Osman et al., 2020) and Cyprus [7.7%] (Hamamy et al., 2013).While the frequency of the same mutation in the present study was lower than that detected in Kuwait [17.6%] (Haider and Adekile; 2005), Bahrin [32%] (Jassim et al., 2001),UAE [45.45%] (El-Kalla and Baysal, 1998), Jordan [43%] (Abu-Ghoush,2008), Saudi Arabia [64%] (Hellani et al., 2009) and Iraq [69%] (Al-Allawi et al., 2009).

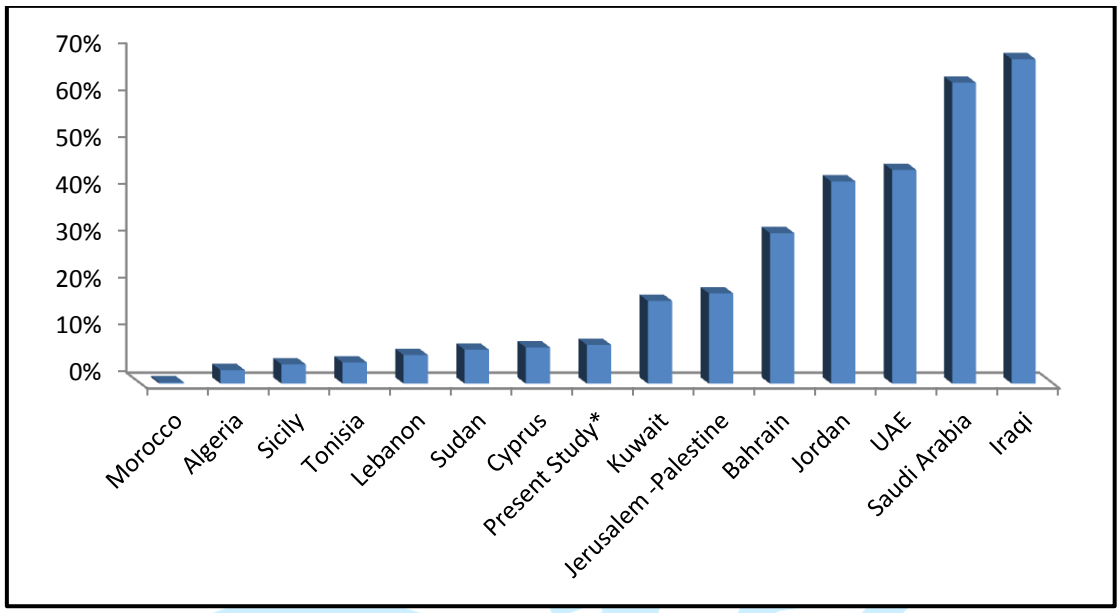

Fig. 1 Comparison of -$\alpha^{3.7}$ allele frequency in the present study and some countries

Relying on the type of mutation and its impact on globin gene expression, the acuteness of anemia differs from minor to severe. For instance, -$\alpha^{3.7}$, as compared to other deletional mutations, results in a minimal phenotype (Harteveld and Higgs 2010). Individuals with only one mutated α- gene don't differ from healthy persons and are recognized only upon molecular investigation (Al- Amodi et al., 2018; Borges et al., 2001). So, not identifying those carriers is expected to raise the incidence of α-thalassemia.

In the current study, no significant reduction in hematological parameters, especially in Hb level, was observed in carriers of one mutant allele (-$\alpha^{3.7}\alpha/\alpha\alpha$). This result may be clarified by the fact that normal persons have four copies of the α-globin encoded by two contiguous homologous genes; α1 (*HBA1*) and α2 (*HBA2*) and loss of one copy does not have detrimental effect on the amount of α-globin produced.

α-Thalassemia is characterized by minor microcytosis and hypochromia, decreased hemoglobin levels and increased erythrocytes number, when compared to hematological parameters of normal individuals. These alterations are more noticeable in homozygous α-thalassemia individuals than in their heterozygous counterparts (Harteveld and Higgs, 2010). As observed in this study there was a decrease in the RBCs number, Hb level, MCV and MCH in -$\alpha^{3.7}\alpha/$-$\alpha^{3.7}\alpha$ genotype, compared to individuals with -$\alpha^{3.7}\alpha/\alpha\alpha$ .This was to be predictable, since the uneven globin synthesis in α-thalassemia leads to reduction in the amount of normal Hb (about 30%–35% of the red cell content) per cell, thus leading to the production of hypochromic and microcytic cells (Higgs, 2009a). When amount of the α-globin chain is insufficient, as in α-thalassemia, there is a compatible increase in "αβ" dimer formation, with β chains competing more productively than δ chains for scant α-globin (Steinberg and Nagel, 2009). Therefore, we find that the hemoglobin value in -$\alpha^{3.7}\alpha/\alpha\alpha$ is slightly higher than those who do not carry this mutation (i.e., $\alpha\alpha/\alpha\alpha$).

-$\alpha^{4.2}$, another type of deletional cause of α^{+} thalassemia, is believed to be less frequent in the Mediterranean region. The -$\alpha^{4.2}$ mutation was detected in one patient (0.25%) in this study. This mutation (-$\alpha^{4.2}$) was not detected in Jordan (Abu-Ghoush, 2008). In Turkey; Karakas et al. described the prevalence of -$\alpha^{4.2}$deletion as 4.2%, and it was detected in one case (1.3%) by Demir et al., but Onay et al. reported that they did not detect any -$\alpha^{4.2}$ deletion (Karakas et al.,2015; Demir et al.,2021 and Onay et al.,2015). Among 51

individuals with unexplained hypochromia and/or microcytosis in Iraq 3.9 % had the -$\alpha^{4.2}$ deletion (Al-Allawi et al., 2009). The prevalence was reported in: Bahrain as 2% (Jassim et al., 2001), Tunisia as 0.9 % (Zorai et al., 2002), 1.2 % in Emirates (El-Kalla and Baysal., 1998) and Malaysia as 0.6% (Wee et al.,2005). This mutation was not found in Sudanese (Osman et al., 2020). The highest frequencies were observed in north Iran [8.73%] (Harteveld and Higgs, 2010).

5. Conclusion

The $-\alpha^{3.7}$ deletion mutation constitutes a common α-thalassemia mutation in the investigated population and should be considered when investigating microcytic hypochromic patients.

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Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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