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Frequency of Alpha Thalassemia in Pregnant Women with Microcytic Hypochromic Anemias

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Abstract

Objective: The objective of this study is to ascertain the frequency of alpha thalassemia in pregnant women present with a microcytic hypochromic blood picture.
Methodology: This descriptive study was conducted at Sir Ganga Ram Hospital and the University of Health Sciences Lahore over a period of one year from 2017-2018, enrolling 190 pregnant women with microcytic hypochromic blood picture confirmed via CBC. HPLC and serum ferritin assessments were performed, followed by GAP-PCR for α-3.7 and α-4.2 gene deletions.
Results: Among 190 pregnant mothers, 149 women (78.4%) were diagnosed with iron deficiency anemia (IDA) due to low serum ferritin levels (<30 ng/dL). Additionally, 20 women (10.5%) had β-thalassemia trait, and 6 women (3.1%) exhibited a co-occurrence of IDA and β-thalassemia trait. For those with serum ferritin levels above 30 ng/mL, GAP-PCR analysis was conducted. Among this subgroup, 5 women (2.6%) were found to have α-3.7 gene deletion, while no case of α-4.2 gene deletion was detected.
Conclusion: Our research emphasizes the high incidence of IDA among expectant mothers. Second, it identifies a 2.6% prevalence of the alpha thalassemia 3.7 gene deletion, indicating that microcytic hypochromic anemia in pregnant women is not solely due to iron deficiency but also involves beta and alpha thalassemia traits. Accurate detection requires tests like Hb-electrophoresis/HPLC and molecular studies.
Key Words: IDA (Iron deficiency anemia), GAP PCR (GAP Polymerase Chain Reaction), βTT (β-thalassemia trait), CBC (Complete Blood Count), HPLC (High Performance Liquid Chromatography).

Introduction

Alpha thalassemia is an autosomal recessive disorder marked by the deficient or absent synthesis of alpha globin chains, leading to microcytic hypochromic anemia. α-thalassemia has four types of clinical forms, two of which are clinically identifiable forms and two are carrier states. The carrier states are phenotypically divided into α thalassemia trait and silent carriers. When there is deletion of single α globin gene, the silent carrier state arises. α thalassemia trait occurs due to the deletion of two α genes.[1] Due to the existence of only single working α globin gene, a medical condition arises known as HbH disease. Hydrops fetalis syndrome is caused by the deficiency of all the four globin genes. Chances of extreme maternal problems increases in pregnancy with this Hb Bart's hydrops fetalis and the fetus generally expires in utero or few months after delivery.[2] Significant health hazards are associated with alpha thalassemia, especially when it appears during pregnancy. Complications include pre-eclampsia, congestive heart failure, deteriorating anaemia, and the possibility of threatening miscarriages are more likely to occur in pregnant women with alpha thalassemia.[3]

The HBA1 and HBA2 genes, which are constituents of the alpha-globin gene cluster on chromosome 16, have distinct roles in hemoglobin production because of variations in mRNA expression and stability. HBA2 mutations are more commonly expressed and have a higher hematological impact than HBA1 mutations, especially among carriers.[4] Deletions like -α3.7 and -α4.2 result from unequal recombination events during meiosis, and depending on the quantity and type of gene deletions, it may result in mild to severe forms of α-thalassemia.[5] Recent epidemiological studies considers the predominance of alpha-thalassemia in Southeast Asia population showed surprisingly significant results. Within Vietnam, the rate is astonishing and dominates at 51.5%. Rate in Malaysia was also notable at 17.3%, along with 20.1% in Thailand, 26.7% in Laos and 39.5% in Cambodia.[6] In Pakistan, 8.3% of the population have the -

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α3.7 variant which is a relatively common deletion of α-thalassemia. The rarest types of the -α 4.2 mutation have been seen to occur at a rate of 0.2%.[7] Other research cites the prevalence of the alpha-thalassemia trait in different parts of Pakistan to be within the range of 0.94% to 2.4%.[8]

For this study, microcytic pregnant women were assessed for the beta thalassemia trait prevalence alongside alpha thalassemia and iron deficiency anemia (IDA). Distinctive diagnosis is very important in case of an underlying hemoglobinopathy diagnosis because iron supplementation will likely not improve the hemoglobin levels. Distinguishing these disorders and providing appropriate care to improve maternal and fetal outcomes requires advanced diagnostic techniques such as hemoglobin electrophoresis, high-performance liquid chromatography (HPLC), molecular methods like GAP-PCR, MLPA, NGS, and others.

Methodology

This descriptive study was conducted at the Department of Hematology, University of Health Sciences Lahore, in collaboration with the Punjab Thalassemia Prevention Program Sir Ganga Ram Hospital Lahore, after approval from ethical review committee. Using a calculated sample size of 190, based on a 95% confidence level, convenient sampling was performed to recruit pregnant women with microcytic hypochromic anemia. Inclusion criteria targeted first-trimester anemic women with low MCV and MCH values (MCV < 76 fl and MCH < 27 pg), while excluding those with anemia of chronic diseases like rheumatoid arthritis, SLE or malignancies. Diagnostic steps included CBC and serum ferritin tests to distinguish iron deficiency anemia (ferritin <30ng/ml). Those with higher ferritin levels and suspected beta thalassemia underwent HPLC for HbA2 analysis; if HbA2 was above 3.5%, a beta thalassemia trait was diagnosed. The remaining samples were analyzed with GAP PCR to identify alpha thalassemia gene deletions as illustrated in figure 1.[9]

During sample collection, informed consent was obtained, and each patient's personal and family history was documented. Blood samples (6 mL total) were collected: 3 mL in an EDTA vial for CBC, peripheral blood smear, HPLC, and DNA studies, and 3 mL in a gel vial for serum ferritin analysis. CBC was performed using a Sysmex XT-1800i hematology analyzer, noting RBC indices (MCV <76 fl and MCH <26 pg). HPLC (Bio-Rad Variant II) quantified HbA2 levels from EDTA whole blood, while serum ferritin were measured from the gel vial's centrifuged serum.

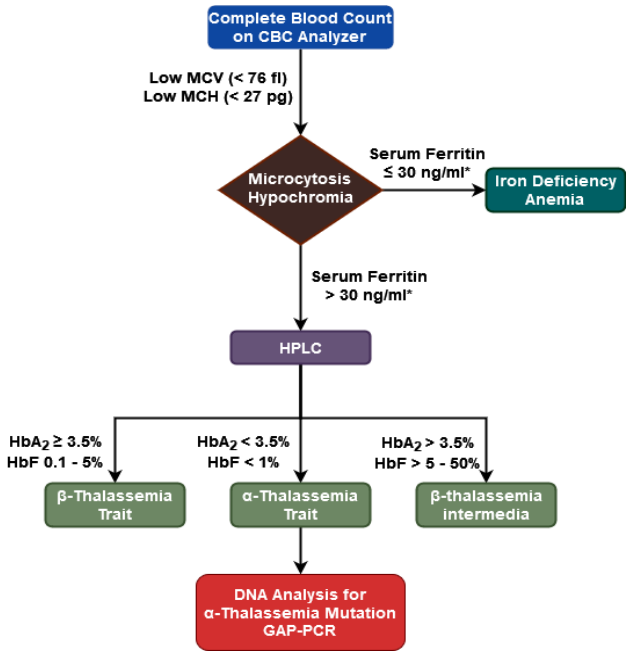

Figure 1. Diagnostic flowchart for the detection of alpha thalassemia.[9]

DNA extraction was performed using the TIANA-amp genomic DNA kit, following a series of steps including the addition of proteinase K, buffer treatments, ethanol mixing, and centrifugation to purify DNA for use in Gap PCR. For Gap PCR, deletional mutations of α-thalassemia were identified by targeting the α 3.7 and α 4.2 gene deletions, utilizing redesigned primers and optimized reagent concentrations. Each reaction mixture contained dNTPs, MgCl2, Q-solution (Qiagen, Hilden, Germany), 2.5 U HotStarTaq DNA polymerase in the supplied reaction buffer (Qiagen) and targeted primers with thermal cycling conditions tailored to amplify the deletion region. The products were analyzed via electrophoresis on a 1% agarose gel to confirm deletion mutations.

Data analysis for the study was performed using SPSS version 20, employing descriptive statistics for qualitative variables and frequency distribution. Quantitative data with a normal distribution was summarized with mean ± SD, while non-normal data used median ± IQR, verified by Shapiro-Wilk's test. Comparisons across groups utilized the independent t-test for normally distributed

data, and the Mann-Whitney U test for non-normal data, with a p-value < 0.05 indicating significance. Ethical considerations included informed consent, where participants were thoroughly briefed on the study's aims, risks, and benefits, and confidentiality was ensured by safeguarding participant identity in study outputs.

Results

This study was conducted with a cohort of 190 pregnant women, all of whom were diagnosed with anemia, ensuring a robust and representative sample for the present research. All the participants were from Lahore, Pakistan. Details of the study group are shown in Table I.

The analyzed hematological attributes included Age, Hemoglobin, MCV, MCH, RBC Count, Hemoglobin A, Hemoglobin A2, Hemoglobin F, and Serum Ferritin. Results showed 78.4% had iron deficiency anemia (IDA) with ferritin below 30 ng/dl, 10.5% had β-thalassemia trait, and 3.1% had both IDA and β-thalassemia trait. GAP-PCR testing revealed alpha thalassemia 3.7 gene deletions in 2.6% of patients, with no cases of alpha thalassemia 4.2 deletion. Additionally, 10 unresolved patients might have been affected by other alpha thalassemia gene deletions (- - SEA, - -FIL, - -MED, - - α 20.5, and - -THAI), which were not encompassed within the scope of this study as illustrated in Figure 2.

Table I: Details of the study group.

Parameters	Details	
Age	18 years – 38 years	
Gender	Male	0
	Female	190
Marital Status	Single	0
	Married	190
Marriage Type	Out of Family Marriage	77 (41%)
	Consanguineous marriage	113 (59%)
Iron Therapy	None	

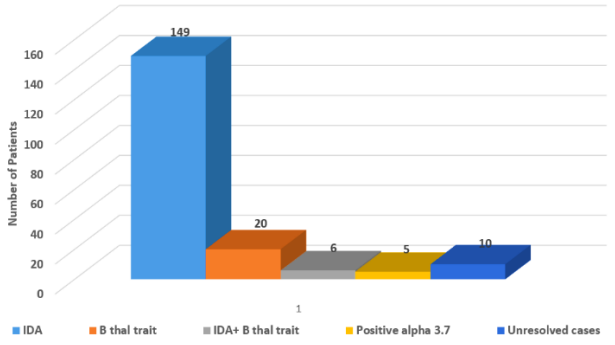

Figure 2. Various causes of microcytic hypochromic Anemia among pregnant women.

The gel photograph revealed the alpha thalassemia 3.7 gene deletion in DNA samples from pregnant women, using a 1 kb DNA ladder for sizing. Distinct 1800 bp bands appeared in lanes 2, 4, and 7, confirming the gene deletion. This pattern aligns with the results shown in figure 3.

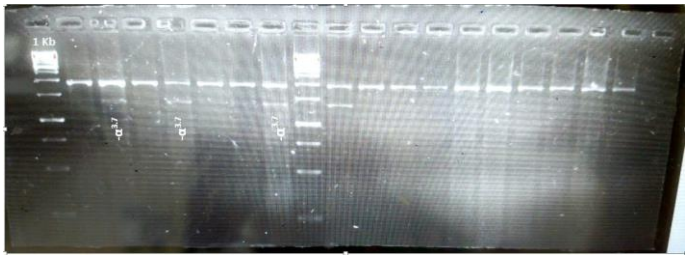

Figure 3. Gel photograph showing bands of Alpha Thalassemia 3.7 gene deletion.

In the analysis of hematological parameters between IDA and β-thalassemia trait, MCV, MCH, RBC Count, Hemoglobin A, Hemoglobin A2 and Serum Ferritin showed significant results. The distinct patterns indicated significantly reduced MCV and MCH, along with an increased RBC count in β-thalassemia trait. Moreover, the elevated levels of HbA2 and Serum Ferritin in the β-thalassemia trait were observed as shown in Table II.

Table II: Comparison of IDA and β-thalassemia trait.

Variables	Diagnosis	Mean ± SD	Median (Q1-Q3)	P value
Age	IDA	27.0 ± 4.7	27.0(23.5-30)	0.822
	β Thal Trait	26.8 ± 3.8	26.0 (24-30)	
Hemoglobin (g/dl)	IDA	8.73 ± 1.1	8.90(8.0-9.5)	0.409
	β Thal Trait	8.98 ± 1.1	9.00(8.3-10.0)	
MCV (fl)	IDA	70.2 ± 6.3	70.4(66.3-75.4)	0.000*
	β Thal Trait	62.8 ± 3.1	62.9(60.5-65.3)	
MCH (pg)	IDA	21.6 ± 2.9	21.7(20.0-23.9)	0.004*
	β Thal Trait	20.2 ± 2.3	20.1(18.7-20.7)	
RBC Count (10^{12}/L)	IDA	4.07 ± 0.4	4.06(3.77-4.37)	0.000*
	β Thal Trait	4.53 ± 0.6	4.60(4.03-5.00)	
Hb A (%)	IDA	97.6 ± 0.4	97.5(97.2-98.0)	0.000*
	β Thal Trait	93.7 ± 1.8	94.0 (93.1-95.0)	
Hb A2 (%)	IDA	2.64 ± 0.4	2.0 (1.7-2.3)	0.000*
	β Thal Trait	5.61 ± 0.8	5.5 (4.9-6.3)	
Hb F (%)	IDA	0.36 ± 0.2	0.40(0.2-0.5)	0.073
	β Thal Trait	0.66 ± 1.7	0.30 (0.1-0.4)	
Serum Ferritin (ng/ml)	IDA	7.19 ± 5.0	5.87(3.9-9.0)	0.000*
	β Thal Trait	46.7 ± 16.5	41.9 (32.5-58.6)	

Table III: Comparison of IDA and Concomitant β-thalassemia trait with IDA.

Variables	Diagnosis	Mean ± SD	Median (Q1-Q3)	P value
Age	IDA	27.0 ± 4.7	27.0 (23.5-30)	0.548
	IDA + β Thal Trait	27.7 ± 5.2	30.5 (22-31)	
Hb (g/dl)	IDA	8.73 ± 1.1	8.90 (8.0-9.5)	0.613
	IDA + β Thal Trait	8.98 ± 0.9	9.10 (8.1-9.8)	
MCV (fl)	IDA	70.2 ± 6.3	70.4 (66.3-75.4)	0.006*
	IDA + β Thal Trait	61.3 ± 6.9	62.1 (54.7-66.0)	
MCH (pg)	IDA	21.6 ± 2.9	21.7 (20.0-23.9)	0.057
	IDA + β Thal Trait	19.1 ± 2.5	19.4 (16.7-20.7)	
RBC Count (10^{12}/L)	IDA	4.07 ± 0.4	4.06 (3.77-4.37)	0.001*
	IDA + β Thal Trait	4.76 ± 0.3	4.91 (4.36-5.01)	
Hb A (%)	IDA	97.6 ± 0.4	97.5 (97.2-98.0)	0.000*
	IDA + β Thal Trait	94.7 ± 0.5	94.7 (94.3-95.2)	
Hb A2 (%)	IDA	2.64 ± 0.4	2.0 (1.7-2.3)	0.000*
	IDA + β Thal Trait	4.96 ± 0.5	5.0 (4.3-5.5)	
Hb F (%)	IDA	0.36 ± 0.2	0.40 (0.2-0.5)	0.173
	IDA + β Thal Trait	0.28 ± 0.1	0.25 (0.2-0.3)	
Serum Ferritin (ng/ml)	IDA	7.19 ± 5.0	5.87 (3.9-9.0)	0.760
	IDA + β Thal Trait	6.7 ± 3.6	8.88 (2.6-9.7)	

While comparing the hematological parameters between IDA cases and patients with both IDA and β-thalassemia trait, significant differences in absolute values of MCV, RCB Count, Hemoglobin A, and Hemoglobin A2 were observed. The distinct patterns indicated significantly reduced MCV, along with an increased RBC count in concomitant β-thalassemia trait with IDA. Additionally, the elevated levels of HbA2 remained a significant feature for concomitant β-thalassemia trait with IDA as shown in Table III.

In the comparison of the same hematological parameters between β-thalassemia trait and alpha 3.7 gene deletion, no significant differences were observed, except for significantly higher Hemoglobin A and Serum Ferritin as well as lower Hemoglobin A2 levels in the case of alpha 3.7 gene deletion as shown in Table IV.

Discussion

Anemia during pregnancy persists as a noteworthy global health challenge, influencing the health of expectant mothers and the well-being of fetuses through various repercussions. The estimated worldwide occurrence of anemia during pregnancy is approximately 41.8%, is indicative of a substantial and persistent public health challenge that demands comprehensive attention.[10]

Iron Deficiency Anemia (IDA) is highly prevalent, with studies indicating rates as high as 70.4% among pregnant women in Pakistan.[11] In our respective research, Of the 190 pregnant women included in the study, IDA was diagnosed in 78.4%, with iron deficiency being a major cause of microcytic hypochromic anemia during pregnancy.

Thalassemia is another anemic condition that increases the possibility of complications for pregnant women, such as heart failure and preeclampsia.[12] This study illustrates the significant variations between IDA and β-thalassemia

Table IV: Comparison of β-thalassemia trait and Alpha 3.7 deletion

Variables	Diagnosis	Mean ± SD	Median (Q1-Q3)	P value
Age	β Thal Trait	26.8 ± 3.8	26.0 (24-30)	0.268
	Alpha 3.7 deletion	28.6 ± 3.3	28.0 (26-31)	
Hb (g/dl)	β Thal Trait	8.98 ± 1.1	9.00 (8.3-10.0)	0.322
	Alpha 3.7 deletion	8.42 ± 1.0	8.60 (7.5-9.2)	
MCV (fl)	β Thal Trait	62.8 ± 3.1	62.9 (60.5-65.3)	0.204
	Alpha 3.7 deletion	65.2 ± 3.5	65.0 (61.9-68.7)	
MCH (pg)	β Thal Trait	20.2 ± 2.3	20.1 (18.7-20.7)	0.475
	Alpha 3.7 deletion	19.9 ± 2.0	20.8 (17.8-21.5)	
RBC Count (10^{12}/L)	β Thal Trait	4.53 ± 0.6	4.60 (4.03-5.00)	0.916
	Alpha 3.7 deletion	4.48 ± 0.9	4.04 (3.76-5.43)	
Hb A (%)	β Thal Trait	93.7 ± 1.8	94.0 (93.1-95.0)	0.001*
	Alpha 3.7 deletion	97.3 ± 0.7	97.2 (96.7-98.0)	
Hb A2 (%)	β Thal Trait	5.61 ± 0.8	5.5 (4.9-6.3)	0.000*
	Alpha 3.7 deletion	2.28 ± 0.6	2.5 (1.6-2.8)	
Hb F (%)	β Thal Trait	0.66 ± 1.7	0.30 (0.1-0.4)	0.336
	Alpha 3.7 deletion	0.38 ± 0.1	0.30 (0.3-0.5)	
Serum Ferritin (ng/ml)	β Thal Trait	46.7 ± 16.5	41.9 (32.5-58.6)	0.021*
	Alpha 3.7 deletion	164.7 ± 71.6	151.55 (101-234.9)	

trait (βTT), indicating that the individuals with βTT had greater red blood cell (RBC) counts, lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), and higher levels of serum ferritin and hemoglobin A2 (HbA2). [13]

Alpha thalassemia 3.7 gene deletion was identified in 2.6% of the study population, which is consistent with earlier studies conducted in Pakistan. Alpha thalassemia 4.2 gene deletion was undetectable, indicating its low incidence. [14] Significant variations in HbA2 and serum ferritin were observed when βTT and alpha thalassemia 3.7 gene deletion were compared; increased HbA2 in βTT has been identified to be a diagnostic indicator.

Different patterns, such as microcytic RBCs and higher HbA2 levels, were seen in cases of co-existing IDA and βTT, indicating that elevated HbA2 is still an essential indicator for βTT even in the presence of IDA.[15] The distinctive patterns observed in individuals with concomitant βTT and IDA indicate significantly reduced MCV, indicating smaller red blood cells. Furthermore, elevated levels of HbA2 remained a significant feature in individuals with concomitant βTT and IDA. This finding supports the notion of our study that elevated HbA2 levels serve as a distinguishing feature for individuals with both conditions similar to the results in the study conducted by P. Sharma.[16] Though concomitant IDA is known to confound Beta thalassemia diagnosis but, in our study, HbA2 levels are still significantly suggestive of beta thalassemia in presence of concomitant IDA. For lowering HbA2 values significantly, the IDA must be extremely severe.

In our study, the prevalence of alpha thalassemia 3.7 gene deletion was determined to be 2.6% among the patients from Lahore indicating its lower occurrence. Conversely, the study targeting Urdu speaking Gujarati and Punjabi populations in Pakistan reported a slightly lower prevalence of 2.5% for the same deletion.[17] The similar frequency of alpha thalassemia 3.7 gene deletion in both studies indicate the potential genetic similarity or shared ancestral background among the populations studied.

However, another study conducted in Pakistan reported a higher frequency of the α3.7 deletion, with a prevalence of 8.3% among the Pakistani population. Alpha thalassemia 4.2 gene deletion was not identified in our research, which is consistent with the results of a study by Shahid et al. in Pakistan that reported a 0.2% prevalence of this particular deletion. These consistent results emphasize the rarity of the alpha thalassemia 4.2 gene deletion in both studies. [14]

Conclusion

In conclusion, our study highlights a number of significant concerns regarding alpha thalassemia in pregnant women with microcytic hypochromic anemia. It first brings focus to the elevated incidence of iron deficiency anemia (IDA) in this population, emphasizing the necessity of regular screening and treatment. Furthermore, this study reveals that the alpha thalassemia 3.7 gene deletion has been identified in 2.6% of expectant mothers.

The significance of other diagnostic tests, including Hb-electrophoresis/HPLC and molecular research, in distinguishing between alpha and beta thalassemia characteristics is highlighted in our study. For an accurate diagnosis, these tests are essential, particularly in cases when iron supplementation is ineffective in treating anemia.

Prenatal care and health consequences for affected women can also be improved by screening partners for thalassemia traits early in pregnancy. This can help with genetic counselling along with informed decisions regarding reproductive health. For improved maternal health, the research offers vital insights into managing different types of anemia in expectant mothers, providing prompt and suitable treatment.

Limitations: One limitation of our research is that we only used GAP PCR to identify deletions in the alpha thalassemia gene. Despite being an effective and well-established method for detecting individual gene deletions, GAP PCR might not be able to detect the total number of deleted genes.

Future Perspective: Alpha thalassemia in pregnant women with microcytic hypochromic anemia will be better understood through long-term studies, improved diagnostic techniques to detect the additional forms of alpha thalassemia gene deletions. Future research should concentrate on investigating different diagnostic methods to enhance the existing use of GAP-PCR, which includes Multiplex Ligation-dependent Probe Amplification (MLPA), real-time PCR assays, and Next-generation sequencing (NGS)

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