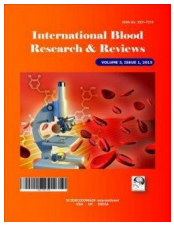

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Early Diagnostics of Beta Thalassemia Minor

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Authors' contributions

This work was carried out in collaboration between both authors. Author ASS designed the study. Author MFBDSHAR wrote the first draft of the manuscript and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Early diagnosis of disease is highly recommended for the treatment purposes by the clinicians. Thalassemia is a genetic disorder which can be inherited from the parents. Thalassemia is classified into two groups alpha thalassemia and beta thalassemia depending upon the severity of the infants. The methods for early diagnosis of beta Thalassamia which is currently used in some diagnostic labs, for instances, current direct and indirect mutation detection method. Recently, most researchers have been discovered the latest or emerging methods to improve the technology in order to minimize invasive methods that may be used as a routine procedure for the future which is better than current methods, like pre-implantation genetic diagnosis and non-invasive prenatal diagnosis. For current methods, chorionic villi sampling (CVS) and amniocentesis are used whereas blastomere biopsy is used for pre-implantation genetic diagnosis. Hence, non-invasive prenatal diagnosis can be perfomed by using fetal cells which is found in maternal plasma such as trophoblasts, erythrocytes and leucocytes. Emerging methods for early diagnosis of beta thalassaemia minor are much safer than current methods that will minimize the risk and less invasive to the patients.

Keywords: Early diagnosis; thalassemia; chorionic villi sampling; amniocentesis.

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1. INTRODUCTION

Thalassemia is a heterogeneous group of genetic disorders of haemoglobin. The causes of thalassemia are due to impaired synthesis of alpha and beta globin chains of haemoglobin. Thalassemia is originated from a Greek word (‘*thalassa’* means sea), which was reported earlier mainly in the Mediterranean population. It was discovered by Thomas Cooley, a paediatrician from Detroit, USA in 1925. Homozygous beta thalassemia or thalassemia major is also known as Cooley anaemia. There are two common types of thalassemia depending upon the lack of synthesis of globin chain such as alpha (α) and beta (β) thalassemia. Alpha thalassemia can be categorized on the basis of clinical severity into 4 types, for example, Hb Bart’s Hydrop’s fetalis syndrome, HbH disease, thalassemia trait and silent carrier. Beta thalassemia, was further sub classified into B-thalassemia major, B-thalassemia intermediate and B-thalassemia minor [1].

1.1 Pathogenesis of Thalassemia

Thalassemia occurs due to genetic defects caused by decrease or absence of particular globin chain production. There are 4 types of genetic defects that can cause thalassemia, single nucleotide mutation, base substitutions, and large deletion within the alpha globin or beta globin clusters and insertion or deletion mutations within the coding region of the mRNA. The single nucleotide mutation caused by some interferes of messenger RNA (mRNA) production that will decrease the amount of mRNA. Base substitutions can cause changes in the function of promoter, RNA processing or mRNA translation as well as modify a codon into nonsense codon that could lead to premature termination of translation or substitution of an incorrect amino acid. Thus, large deletion with alpha and beta globin clusters by removing one or two gene can cause few changes in regulation of the remaining genes in the clusters. Lastly, the occurrence of insertion or deletion mutation of globin chain may create frame shifts to prevent synthesis of complete normal globin polypeptide. The molecular lesions in thalassemia are complex. The most common cause of beta thalassemia reported by point mutation whereas alpha thalassemia caused by gene deletions [2].

1.2 Diagnosis of Thalassemia

Thalassemia patient can be diagnosed by examining the blood smear and measuring of haemoglobin, haematocrit, red cell count, complete blood count (CBC) and red cell indices [2], followed by using Hb electrophoresis or high performance liquid chromatography for quantifying HBA2 if MCV and MCHC as low [2,3,4]. If HBA2 is more than 3.5%, patient may categorised under beta thalassemia trait, delta beta thalassemia and Hb Lepore trait. If HBA2 is less than 3.5%, it is the alpha thalassemia trait and needs to perform further test for DNA analysis [5]. Other special procedures can be performed by using mass spectrometry. Mass spectrometry can be used to assess the mass of the globin chains by detecting single amino acid substitutions in the globin chain and identify various haemoglobin molecules [6]. Antenatal diagnosis is also used to detect patient’s genetic mutation [7].

2. SYNTHESIS OF GLOBIN

Alterations in the sites of erythropoiesis are related to alterations in the type of haemoglobin produced. The synthesis of haemoglobin is maintained by two multigene clusters on chromosome 16 which is encoding of alpha like globin whereas chromosome 11 encodes beta-like globins [8]. The synthesis of globin occurs in three stages, transcription, processing of mRNA and translation as shown in Fig. 1. DNA polymerase enzyme is needed for the synthesis of a single strand of RNA which is originated from DNA template. The base sequence of RNA is complementary to the base sequence of transcription. The promoter will bind to the RNA polymerase for the beginning of translation. RNA molecule assembled when RNA polymerase move along the DNA strand in a 5’ to 3’ direction. The transcription may proceed through exons and intron. RNA polymerase will be isolated from the DNA strand when comes into contact with chain terminating sequence. RNA strands have been formed known as messenger RNA (mRNA) [8]. This process is followed by the processing of mRNA by addition of a cap structure and poly-A-tail and by the removal of introns. Cap structure is attached at 5’ end of mRNA for beginning of translation. Polyadenylation is needed for stability of the transcript and its transport to the cytoplasm. AAUAAA sequence at the 3’ end alerts the addition of poly-A tail about 20 bases downstream from the polyadenylation site. The removal of introns and attached together of exons in the mRNA transcript are prerequisite before mRNA is transported from the nucleus to the cytoplasm [8].

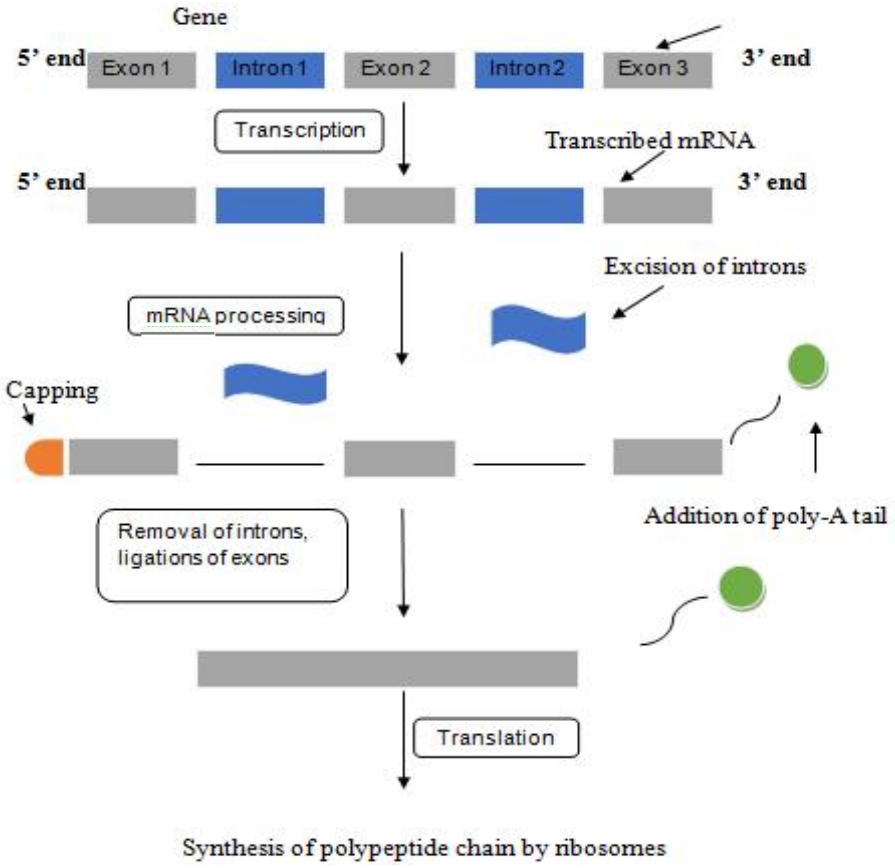

Fig. 1. Globin chain synthesis [9]

Translation takes place in ribosomes to synthesis polypeptide chain according to the directions supplied by mRNA template. Messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA) are involved in the synthesis of polypepetides. The DNA template will be transcribed into mRNA where transfer of genetic code occurs from the nucleus to the cytoplasm of erythroblasts to regulate the sequence of amino acids in the formation of polypeptide. Transfer RNA plays an important role where specific amino acids transported from cytoplasm to the specific locations (codons) along with mRNA strand. After translation process and synthesis of polypeptide chain by ribosomes, it transform into globin chain.

Globin gene expression may alter during development to build different haemoglobin tetramers such as embryonic such as Hb Gower I (ζ2ε2), Hb Gower 2 (α2ε2) and Hb Portland (ζ2γ2), Hb fetal (α2γ2) and adult Hb A (α2β2) and Hb A2 (α2δ2). An appearance of clinical manifestation in hemoglobinopathies plays an important role in developmental alteration of globin genes expression. Alpha thalassemia can be identified at birth whereas beta thalassemia can be detected a few months after the birth [8].

3. EPIDEMIOLOGY

Statistical data of the number of patients with alpha and beta thalassemia in seven regions are shown in Fig. 2. Beta thalassemia has the highest number in Asia and South East Asia, reaching 32665 patients and 21693 patients respectively**.** Beta thalassemia in Eastern Mediterranean has higher number than Pacific region about 9716 patients. Alpha thalassemia is also high in Asia region approximately 17708 patients, followed by Pacific region were 8267 patients. African region has high number of beta thalassemia about 1520 patients, followed by American and Europe region about 534 and 498 patients respectively. Alpha thalassemia has the lowest number in African, with 11 patients and Eastern Mediterranean about 1 patient only [10].

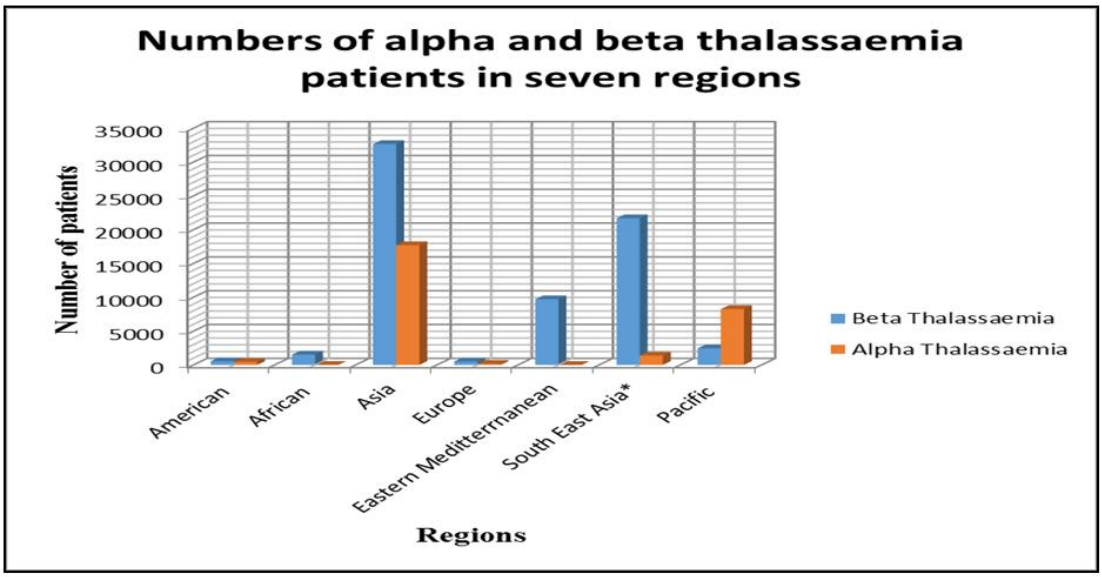

Fig. 2. Number of patients with different types of thalassemia in seven regions [11]

4. DIAGNOSTIC METHODS

Diagnosis of disease is a main challenge for clinicians. It is always recommended that early diagnostics is always better to start treatment and preventive measures for disease.

4.1 Prenatal Diagnosis

Prenatal diagnosis is a method used to monitor human couples and either if these patients have genetic disorder. It also helps them to detect the patient's condition in order to avoid any risk to a child affected with severe haemoglobin disorder. There are four basic parameters of prenatal diagnosis for thalassemia such as timely discovering of couples at risk for an affected pregnancy, the description of their disease causing mutation, acquire the fetal material properly and examine the genotype of the fetal DNA on the basis of parental mutation. Recently, the developments of an early diagnostic are designed and it has been routinely used in many countries. Researchers had discovered the new methods to diagnose and determine the haemoglobin disorder on a prenatal. Moreover, some researchers had discovered the latest version of genetic diagnosis for haemoglobin disorders such as pre-implantation genetic diagnosis and non-invasive prenatal diagnosis which can contribute with some major advantages to the patient with thalassemia disease [12]. For current methods, chorionic villi sampling (CVS) and amniocentesis are routinely use for the detection of genetic disorder. Chorionic villi sampling can be collected when the patient is 10 to 12 weeks of pregnancy whereas amniocentesis can be collected after 15^{th} weeks of pregnancy [12].

4.2 Current Direct Mutation Detection Methods

There are two types of current methods being used in the research lab such as current direct and indirect mutation detection methods. Current direct mutation detection methods are type of tests used to detect specific known mutation in the population group and mutation detection in carriers of beta thalassemia [13,14], for example, Reverse dot blot and Amplification Refractory mutation system (ARMS) [11]. The researchers have found reverse dot blot targeted only for common mutation of a peculiar ethnic group in Thailand and cause some difficulties to detect less common mutation. Thus, they had created a reverse dot blot strip for the 10 beta-thalassemia mutations, including beta-thalassaemic hemoglobinopathies Hb E and Hb Malay that had been reported about ninety six percent of beta thalassemia in Thailand and other strip for six less common Thai mutations. The second strip avoids requirement of more technically challenging methods. They made multiple copies and identifiable beta globin DNA like two shorter fragments that include all known Thai mutations in order to keep from happening trouble involve with secondary structure of amplified full-length

target DNA [9]. Reverse dot-blotting is a rapid method, specific for identification of beta-thalassemia mutations. Amplification refractory mutation system (ARMS) can be used to identify the presence of small deletions of the beta-globin gene and doubt the truth of a very high HbA2 levels if a mutation is not discovered by DGGE analysis [15,16].

4.3 Current Indirect Mutation Detection Methods

Current indirect mutation detection methods are used to examine for the presence of genes regions to identify of sequence variation within each region and specific for unknown mutation after family studies [11,12]. Denaturing Gradient Gel Electrophoresis (DGGE) and denaturing High-Performance Liquid Chromatography (dHPLC). Researchers suggested that DGGE is better than Polymerase chain reaction (PCR) amplified beta globin DNA since they had encountered some problem with Allelic-specific oligonucleotide ASO probes." PCR amplified genomic DNA showed a very diverse spectrum of beta thalassemia in Netherlands with the result about 20% of the beta thalassemia mutation cannot be identified with this problem. DGGE was preferred and they made multiple copies with certain regions of the beta globin gene. On the basis of reported result researchers concluded that several of these mutations to specific regions of the gene rapidly which they were again amplified and sequenced promptly [17]. Denaturing high-performance liquid chromatography (dHPLC) is a method that are applied to identify of known and unknown point mutations which is highly sensitivity and dependable. Coupling heteroduplex and primer extension analysis on the basis of dHPLC is highly specific for beta-thalassemia identification [18,19]. Researchers have developed dHPLC where different compound with diverse in character, identical alleles of human beta globin (HBB) gene mutation and heteroduplex elution profile were detected. Somehow, they also had developed by combining dHPLC and multiplex primer extension analysis for the genotyping of common disease causing mutations in the HBB gene [20]. In Southern Italy, the researchers have done this by using dHPLC where direct sequence analysis was utilized to identify common and rare mutations within beta globin gene. It even identified beta globin gene mutation if standard mutation detection method failed to reveal the result. By this, parental chromatography is dependable because it is suitable for genetic screening of prenatal and postnatal individual to diagnose beta thalassemia [21].

4.4 Emerging Detection Methods

The examples of emerging detection methods are Multiplex Ligation-dependent Probe Amplification (MLPA) and Real-time PCR. Mutilplex ligation-dependent probe amplification (MLPA) is an emerging detection method that are able to detect duplicate number genomic variant within a targeted region, designated replacement for Southern blot analysis and Fluorescene in situ hybridization (FISH) or second method to gap-PCR in order to examine known and unknown deletions that can cause alpha, beta or delta beta-thalassemia. MLPA is mainly on ligation of multiple probe-pairs combined across a region of interest. The universal-tag PCR primers are used with quantitative PCR to make multiple copies of all ligated probe pairs. It allows fragments analysis of PCR products [22,23]. The usage of PCR primers were carried out with ligated probe pairs in PCR steps which are semi-quantitative and permit to identify deletions or duplications across the locus. The advantages of MLPA is amenable, well regulated and make your own oligonucleotide probe-pairs. The utilization of probe pair with varied lengths, dissimilar tags and fluorescent labels can investigate 50 probe sets across a large region of interest. Other than that, commercial kits are readily available for identifying copy number variations across alpha and beta globin gene clusters, for example: MRC-Holland and Service ^{XS} available in most diagnostic laboratories but it is not common for prenatal diagnosis. Real-time PCR combine with microvolume rapid-cycle PCR with fluorometry that will enable the real-time fluorescent checking of the amplification reaction for measuring by the quantity of PCR or describing the qualities of PCR products or rapid investigate of genotype, blocking the action of manipulating of any-post–PCR sample. The identifications potential sequences to investigate the genotype applications such as single nucleotide polymorphisms, utilizes the use of two fluorescent probes which combine to near internal sequences within target amplified DNA, regions expected to contain the mutations [24,25].

4.5 Pre-implantation Genetic Diagnosis

Pre-implantation genetic diagnosis is the most common genetic material currently obtained from

blastomere biopsy. Oocyte or zygote biopsy can be performed for genetic analysis. The example of pre-implantation genetic diagnosis is Mini sequencing or real-time PCR. This method is capable to discover of the mutation which is rapid and free from error. It is linked to Single Nucleotide polymorphism (SNPs) for the accuracy of the PGD. The amplification of small DNA is more productive with minimum effort than the larger fragment of DNA when beginning from a single cell. Minisequencing is suitable for single cell PCR and combined with microcapillary systems which are used for disease mutation analysis and informative linked SNPs [26]. The methods that are used for genotyping single cells for PGD with rapid and accurate result are Real-time PCR with hybridization probes. It is appropriate for PGD of Beta–thalassaemic hemoglobinopathies because of minute size of the beta gene that allows multiplex genotyping in some cases [27].

4.6 Non–invasive Prenatal Diagnosis

Researchers have reported in their studies that fetal cells such as trophoblasts, erythrocytes and leucocytes can be found in maternal cells [28,29,30]. Beginning from 1960s to 1990s, modus operandi was examined for the separation of fetal nucleated cells from maternal plasma [31,32,33]. The example of non-invasive prenatal diagnosis is Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) related to conventional homogenous Mass EXTEND (hME) assay or a nucleotide specific single allele base extension reaction (SABER) assay, possible for identification of fetal-specific alleles in maternal plasma [15,34,35,36]. This was applied to prevent the occurrence of the fetal inheritance of the four most prevalence Southeast Asian beta-thalassemia mutations in at-risk pregnancies within 7 to 21 gestation. Fetal haplotype analysis was related to single polymorphism joined beta-globin locus, HBB in maternal plasma is capable to exhibit the result which is suitable for couples sharing identical mutations [37]. Mass spectrophotometer is vulnerable to high output automated analysis. The disadvantages of these methods are expertise is needed to handle complication of method and high cost to purchase automated analysis in some diagnostic or research laboratories.

5. CONCLUSION

Thalassemia is a genetic disorder which is inherited from the parents. Thalassemia can be classified into two groups; alpha and beta thalassemia depends on the severity of the infants. Some infants cannot survive if it is severe thalassemia but some infants may survive with asymptomatic (carrier). There are some lab diagnostics that can be detect thalassemia by using different techniques for early diagnosis such as current method which is frequently used in the laboratory nowadays. Some researchers had discovered that the latest or emerging methods to improve the technology in order to minimize invasive methods that may be used as routine procedure for the future which is better than current methods.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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