

DIAGNOSTIC FOR BETA THALASSEMIA MINOR

ABSTRACT

Early diagnosis of disease is highly recommended for the treatment purposes by the clinicians. Thalassemia is a genetic disorder which is inherited from the parents. Thalassemia is classified into two groups alpha thalassemia and beta thalassemia depending upon the severity of the infants. Some infants cannot survive if it is severe thalassemia but some infants may survive with asymptomatic thalassemia. There are some lab diagnostics that can be detected by using different technique for early diagnosis such as current method which is frequently used in the laboratory nowadays. Researchers have been 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.

Keywords: [Diagnostic, thalassemia, blood]

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].

I-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].

II-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 [5]. This is followed by using Hb electrophoresis or high performance liquid chromatography for quantifying HBA2 if MCV and MCHC as low [5, 7, 8]. 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 [6]. 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 [9]. Antenatal diagnosis is also used to detect patient's genetic mutation [10].

III-Treatment of Thalassemia

Treatment is not required for beta Thalassemia minor which is asymptomatic. Treatment can be applied to the patient who has beta Thalassemia major. Blood transfusion can be given to the patient for severe Thalassemia. Unfortunately, excessive of blood transfusion can cause iron accumulation in the heart and liver that could lead to cardiac dysfunction and cirrhosis. The proper treatment to remove the excess of iron from the body is to use iron chelation therapy. Deferoxamine and deferasirox are an example for iron chelation therapy. Folic acid supplements can be used to build healthy red blood cells. Last but not least, bone marrow stem cells can be transplant and replace with healthy stem cells from the donors in order to cure Thalassemia for young patients [3, 4].

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 [11]. The synthesis of globin occurs in three stages, transcription, processing of mRNA and translation as shown in **figure 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

84 sequence. RNA strands have been formed known as messenger RNA (mRNA)
 85 [11]. This process is followed by the processing of mRNA by addition of a cap
 86 structure and poly-A-tail and by the removal of introns. Cap structure is attached at
 87 5' end of mRNA for beginning of translation. Polyadenylation is needed for stability
 88 of the transcript and its transport to the cytoplasm. AAUAAA sequence at the 3' end
 89 alerts the addition of poly-A tail about 20 bases downstream from the
 90 polyadenylation site. The removal of introns and attached together of exons in the
 91 mRNA transcript are prerequisite before mRNA is transported from the nucleus to
 92 the cytoplasm [11].

93 Translation takes place in ribosomes to synthesis polypeptide chain according to the
 94 directions supplied by mRNA template. Messenger RNA (mRNA), transfer RNA
 95 (tRNA) and ribosomal RNA (rRNA) are involved in the synthesis of polypeptides.
 96 The DNA template will be transcribed into mRNA where transfer of genetic code
 97 from the nucleus to the cytoplasm of erythroblasts and regulate the sequence of
 98 amino acids in the formation of polypeptide. tRNA plays an important role where
 99 specific amino acids transport from cytoplasm to the specific locations (codons)
 100 along mRNA strand. After translation process and synthesis of polypeptide chain by
 101 ribosomes, it transform into globin chain. Globin gene expression may alter during
 102 development to build different haemoglobin tetramers such as embryonic such as Hb
 103 Gower I ($\zeta 2\epsilon 2$), Hb Gower 2 ($\alpha 2\epsilon 2$) and Hb Portland ($\zeta 2\gamma 2$), Hb fetal ($\alpha 2\gamma 2$) and
 104 adult Hb A ($\alpha 2\beta 2$) and Hb A2 ($\alpha 2\delta 2$). An appearance of clinical manifestation in
 105 hemoglobinopathies plays an important role in developmental alteration of globin
 106 genes expression. Alpha Thalassemia can be identifying at birth whereas beta
 107 Thalassemia can be detect a few months after the birth [11].

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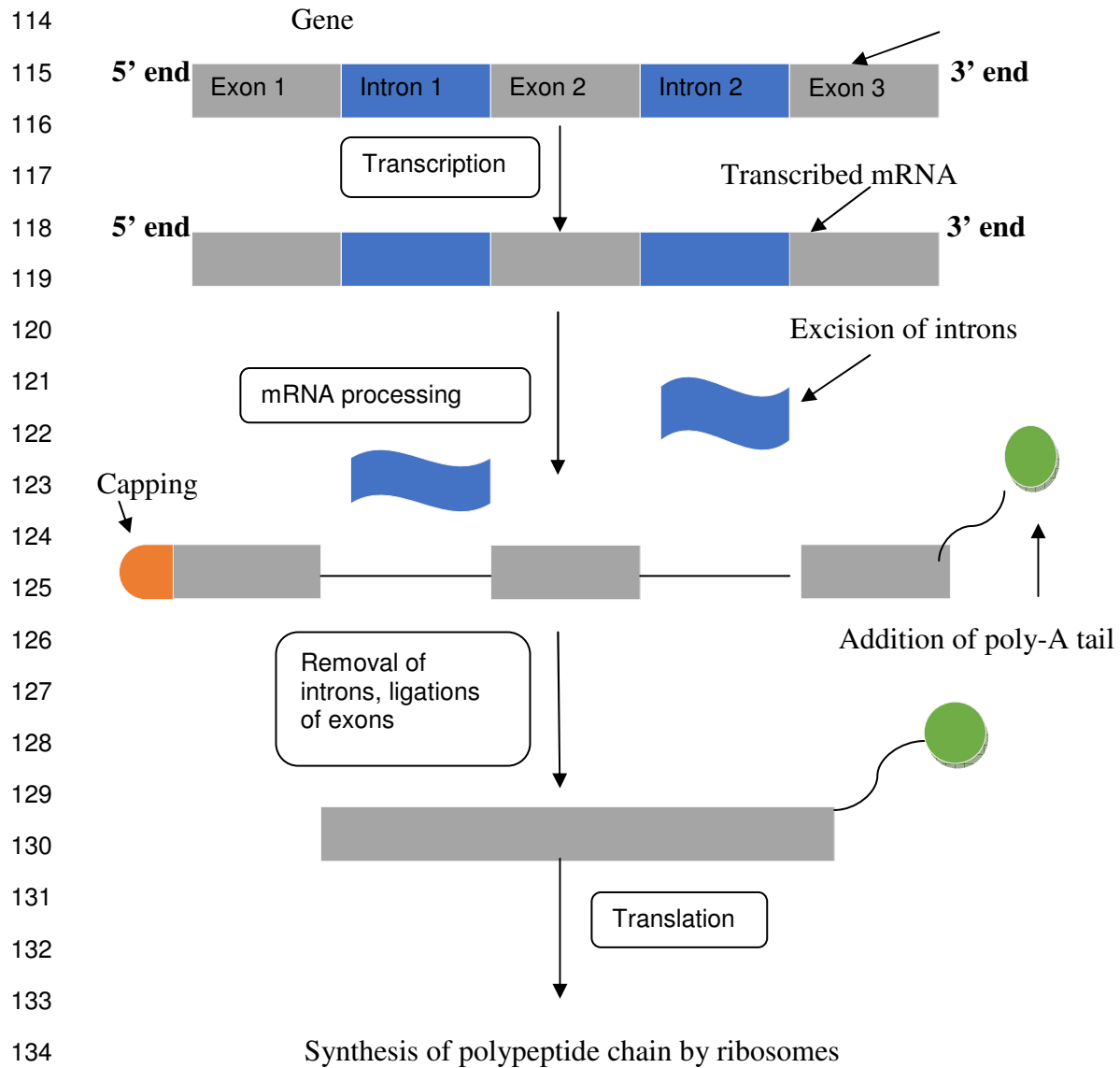
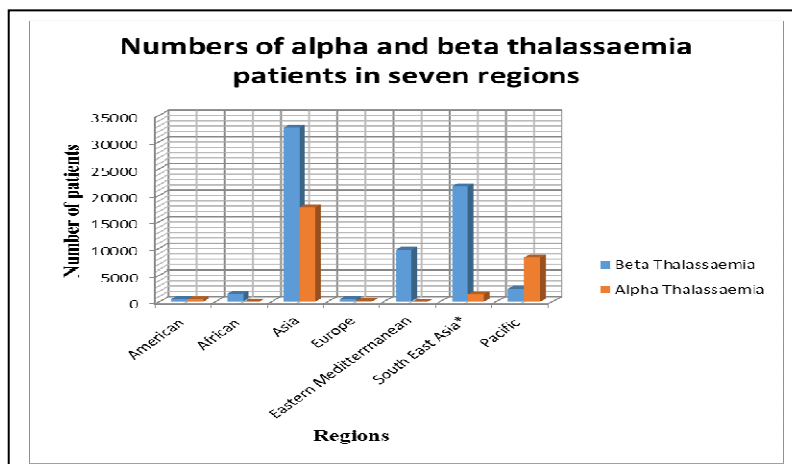


Figure 1: Globin chain synthesis

3. EPIDEMIOLOGY



144 **Figure 2: Number of patients with different types of Thalassemia in seven**
 145 **regions.**

146 Statistical data of the number of patients with alpha and beta Thalassemia in seven
 147 regions are shown in figure 2. Beta Thalassemia has the highest number in Asia and
 148 South East Asia, reaching 32665 patients and 21693 patients. Beta Thalassemia in
 149 Eastern Mediterranean has higher number than Pacific region about 9716 patients.

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151 **Figure 2: Number of patients with different types of Thalassemia in seven**
 152 **regions**

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154 Alpha Thalassemia is also high in Asia region approximately 17708 patients,
 155 followed by Pacific region were 8267 patients. African region has high number of
 156 beta Thalassemia about 1520 patients, followed by American and Europe region
 157 about 534 and 498 patients. Alpha Thalassemia has the lowest number in African,
 158 with 11 patients and Eastern Mediterranean about 1 patient [12].

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160 **4. DIAGNOSTIC METHODS**

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

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165 **I-Prenatal diagnosis**

166 Prenatal diagnosis is a method used to monitor a human couples and either if these
 167 patients have genetic disorder. It also helps them to detect the patient's condition in
 168 order to avoid any risk to a child affected with severe haemoglobin disorder. It has
 169 been 30 years of experience to develop a new method of prenatal diagnosis for
 170 haemoglobin disorders on the basis of molecular analysis or amniocyte DNA since
 171 1970s. There are four basic parameters of prenatal diagnosis for Thalassemia such as
 172 timely discovering of couples at risk for an affected pregnancy, the description of
 173 their disease causing mutation, acquire the fetal material properly and examine the

174 genotype of the fetal DNA on the basis of parental mutation. Nowadays, the
 175 developments of early diagnostic are designed and it has been routinely used in many
 176 countries for the current methods but the researchers had discovered the new
 177 methods to diagnose and determine the haemoglobin disorder on a prenatal.
 178 Moreover, some researchers had discovered the latest version of genetic diagnosis
 179 for haemoglobin disorders such as pre-implantation genetic diagnosis and non-
 180 invasive prenatal diagnosis which can contribute with some major advantages to the
 181 patient with Thalassemia disease as well [13]. For current methods, chorionic villi
 182 sampling (CVS) and amniocentesis are routinely use for the detection of genetic
 183 disorder. Chorionic villi sampling can be collected when the patient is 10 to 12
 184 weeks of pregnancy whereas amniocentesis can be collected after 15th weeks of
 185 pregnancy [13].

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187 **II- Current direct mutation detection methods**

188 There are two types of current methods being done in the research lab such as current
 189 direct and indirect mutation detection methods. Current direct mutation detection
 190 methods are type of tests used to detect specific known mutation in the population
 191 group and mutation detection in carriers of beta Thalassemia [37, 38], for example,
 192 Reverse dot blot and Amplification Refractory mutation system (ARMS) [15]. The
 193 researchers have found reverse dot blot targeted only for common mutation of a
 194 peculiar ethnic in Thailand and cause some difficulties to detect less common
 195 mutation. Thus, they had created a reverse dot blot strip for the 10 beta-Thalassemia
 196 mutations, including beta-thalassaemic hemoglobinopathies Hb E and Hb Malay that
 197 has reported about ninety six percent of beta Thalassemia in Thailand and other strip
 198 for six less common Thai mutations. The second strip avoids require for more
 199 technically challenging methods. They made multiple copies and identifiable beta
 200 globin DNA like two shorter fragments that include all known Thai mutations in
 201 order to keep from happening trouble involve with secondary structure of amplified
 202 full-length target DNA [14]. Reverse dot-blotting is a quick method, specific for
 203 identifying 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 [39].

III- 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 [37, 38], for instances, Denaturing Gradient Gel Electrophoresis (DGGE) and denaturing High-Performance Liquid Chromatography (dHPLC). Some researchers suggest 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. By this, they found out that it assign several of these mutations to specific regions of the gene rapidly which they were again amplified and sequenced promptly [16]. 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 [17, 18]. Some 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 [19]. Another case 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 [20].

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237 **IV-Emerging detection methods**

238 The examples of emerging detection methods are Multiplex Ligation-dependent
239 Probe Amplification (MLPA) and Real-time PCR. Multiplex ligation-dependent
240 probe amplification (MLPA) is an emerging detection method that are able to detect
241 duplicate number genomic variant within a targeted region, designated replacement
242 for Southern blot analysis and FISH or second method to gap-PCR in order to
243 examine known and unknown deletions that can cause alpha, beta or delta beta-
244 Thalassemia. MLPA is mainly on ligation of multiple probe-pairs combined across a
245 region of interest. The universal-tag PCR primers are used with quantitative PCR to
246 make multiple copies of all ligated probe pairs. By this, it allows fragments analysis
247 of PCR products [21, 22]. The usage of PCR primers will be carrying out with
248 ligated probe pairs in PCR steps which are semi-quantitative and permit to identify
249 deletions or duplications across the locus. The advantages of MLPA is amenable,
250 well regulated and make your own oligonucleotide probe-pairs. The utilization of
251 probe pair with varies lengths, dissimilar tags and fluorescent labels can investigate
252 50 probe sets across a large region of interest. Other than that, commercially kits are
253 readily available for identifying copy number variations across alpha and beta globin
254 gene clusters, for example: MRC-Holland and Service^{XS}. It is now available in most
255 diagnostic laboratories but it is not common for prenatal diagnosis. Real-time PCR
256 combine with microvolume rapid-cycle PCR with fluorometry that will enable the
257 real-time fluorescent checking of the amplification reaction for measuring by the
258 quantity of PCR or describing the qualities of PCR products or rapid investigate of
259 genotype, blocking the action of manipulating of any-post-PCR sample. The
260 identifications potential sequences for investigate the genotype applications such as
261 single nucleotide polymorphisms, utilizes the use of two fluorescent probes which
262 combine to near internal sequences within target amplified DNA, one of the cover
263 the regions expected to contain the mutations [23, 24].

264 **V-Pre-implantation genetic diagnosis**

265 For pre-implantation genetic diagnosis, the most common genetic material currently
 266 obtained from blastomere biopsy. Oocyte or zygote biopsied can be performing for
 267 genetic analysis. The example of pre-implantation genetic diagnosis is Mini
 268 sequencing or real-time PCR. This method is capable to discover of the mutation
 269 which is rapid and free from error. It is linked to Single Nucleotide polymorphism
 270 (SNPs) for the accuracy of the PGD. The amplification of small DNA is more
 271 productivity with minimum wasted effort than the larger fragment of DNA when
 272 beginning from a single cell. Minisequencing is suitable for single cell PCR and
 273 combined with microcapillary systems which are used for disease mutation analysis
 274 and informative linked SNPs [25]. The methods that are used for genotyping single
 275 cells for PGD with rapid and accurate result are Real-time PCR with hybridization
 276 probes. It is appropriate for PGD of Beta-thalassaemic hemoglobinopathies because
 277 of minute size of the beta gene that allows multiplex genotyping in some cases [26].

278

279 **VI-Non-invasive prenatal diagnosis**

280 Researchers have reported in their studies that fetal cells such as trophoblasts,
 281 erythrocytes and leucocytes can be found in maternal cells [27, 28, 29]. Beginning
 282 from 1960s to 1990s, modus operandi was examined for the separation of fetal
 283 nucleated cells from maternal plasma [30, 31, 32]. The example of non-invasive
 284 prenatal diagnosis is Matrix-assited laser desorption ionization time of flight mass
 285 spectrometry (MALDI-TOF MS) related to conventional homogenous Mass
 286 EXTEND (hME) assay or a nucleotide specific single allele base extension reaction
 287 (SABER) assay, possible for identifying of fetal-specific alleles in maternal plasma
 288 [33, 34, 35] and this application is being done to prevent the occurrence of the fetal
 289 inheritance of the four most prevalence Southeast Asian beta-Thalassemia mutations
 290 in at-risk pregnancies within 7 to 21 gestation. Fetal haplotype analysis related to
 291 single polymorphism joined beta-globin locus, HBB in maternal plasma is capable to
 292 exhibit the result which is suitable for couples sharing identical mutations [36]. Mass
 293 spectrophotometer is vulnerable to high output automated analysis. Unfortunately,

294 the disadvantages are complicated to handle and unaffordable to purchase this
295 automated analysis in some diagnostic or research laboratories.

296

297 **CONCLUSION**

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

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