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Relationship of Reproductive Hormones and Fertility in Patients with Beta-Thalassemia Major

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Abstract: β-thalassaemia major (BTM) has a high prevalence worldwide and is associated with considerable morbidity and mortality. The aim of this review is to provide an illustrative overview of the reproductive health and pregnancy related issues in females with β-thalassaemia. A review search was performed in four international databases (1980-2018) to identify the potentially relevant articles. Common reproductive health disorders are hypo-gonadotrophic hypogonadism, infertility, delayed or absent sexual development, diabetes, hypothyroidism, hypoparathyroidism, osteopenia, preeclampsia, gestational hypertension, polyhydramnios, oligohydramnios, thrombosis, renal failure, peripheral vascular resistance, placenta previa, pleural effusion and pulmonary hypertension. Many of those aspects are related to iron overload and to ineffective erythropoiesis. Foetal complications include neural tube defects, abnormalities in different organs, spontaneous abortion, foetal loss, preterm birth, foetal growth restriction and low birth weight. Antenatal screening and accurate genetic prenatal examinations are effective measures for early diagnosis of thalassaemia and a detailed plan for management of pregnancies in BTM is important for favourable maternal and foetal outcome.

Key words: Beta-thalassemia, reproductive hormones, menstrual abnormalities.

1.1 Female Reproductive System: Anatomy

The human female reproductive system is one of the most vital parts of human reproductive process. It is composed of the internal and external genitalia. The external genitalia consist of the vulva which comprises the labia majora, labia minora, clitoris, vestibular bulbs monsveneris (pubis), urethral and periurethral gland ducts. The internal genitalia contain the vagina, cervix, uterus, fallopian tubes and ovaries. These organs are protectively located deep within the body [1, 2].

2. The Female Reproductive Organs

The vagina is a hallow fibro muscular canal lined with stratified squamous epithelium. It extends from the vulvar vestibules to the uterus. It is longer in the posterior wall (around 9 cm) than anteriorly (approximately 7 cm), the opening of the vagina may be covered by a membrane or surrounded by a fold of connective tissue called hymen, this tissue is usually replaced by irregular tissue tags later in life as sexual activity and child birth occur. The vagina acts as the receptacle for the male's sperm and it is a part of the birth canal (through which the fetus passes during delivery) [3, 4].

3. Physiology

The main functions of genital organs include the following: (a) produce ova; (b) secret sex hormones; (c) receive the male spermatozoa during sexual intercourse; (d) protect and nourish the fertilized egg until it is fully developed; (e) deliver the fetus through the birth canal; (f) provide nourishment to the neonate by the milk which has been secreted by mammary glands in the breasts [1, 5].

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The Reproductive Cycle in Fertile Female:

Oogenesis

Oogenesis is the process of developing an ovum (male equivalent is spermatogenesis) [6, 7]. The first part of oogenesis starts in the germinal epithelium, which gives rise to the development of ovarian follicles, the functional unit of the ovary. Oogenesis consists of several sub-processes oocytogenesis, ootidogenesis, and finally maturation to form an ovum (oogenesis proper). Folliculogenesis is a separate sub-process that accompanies and supports all three oogenetic sub-processes [8, 9].

A. Oocytogenesis

Oogenesis starts with the process of developing oogonia, which occurs via the transformation of primordial follicles into primary oocytes, a process called oocytogenesis, which is complete either before or shortly after birth [10, 11]. It is commonly believed that, when oocytogenesis is complete, no additional primary oocytes are created. In contrast to the male process of spermatogenesis, where gametocytes are continuously created, in other words, primary oocytes reach their maximum development at about 20 weeks of gestational age, when approximately seven million primary oocytes have been created; however, at birth, this number has already been reduced to approximately 1-2 million [10, 12, 13].

B. Ootidogenesis

The succeeding phase of ootidogenesis occurs when the primary oocyte develops into an ootid. This is achieved by the process of meiosis [14]. In fact, a primary oocyte is, by its biological definition, a cell whose primary function is to divide by the process of meiosis [15]. However, although this process begins at prenatal age, it stops at prophase I. In late fetal life, all oocytes, still primary oocytes, have halted at this stage of development called the dictyate. After menarche, these cells then continue to develop, although only a few do so every menstrual cycle. Just before ovulation, the first meiotic division is completed. One of the daughter cells (secondary oocyte) receives most of the cytoplasm, while other (the first polar body) fragments and disappears. The secondary oocyte immediately begins the second meiotic division to form ootid and second polar body, but this division stops at metaphase and is completed only when a sperm penetrates the oocyte [16].

C. Maturation into Ovum:

Both polar bodies disintegrate at the end of meiosis II, leaving only the ootid, which then eventually undergoes maturation into a mature ovum. The function of forming polar bodies is to discard the extra haploid sets of chromosomes that have resulted as a consequence of meiosis [17].

D. Folliculogenesis:

Synchronously with ootidogenesis, the follicle cells surrounding the ootid have developed from a primordial follicle to a preovulatory one [18, 19].

The hypothalamus-Pituitary-Gonadal Axis:

The hypothalamus-pituitary-gonadal axis consists of a closed loop feedback control mechanism directed at maintaining normal reproductive functions. The pulsatile secretion of gonadotropins releasing hormone (GnRH) appears to be essential for stimulatory effects on luteinizing hormone (LH) and follicle stimulating hormone (FSH) released from anterior pituitary gland [20, 21].

Menstrual Cycle:

The menstrual cycle (from puberty to the menopause) is a complex event which depends on interactions between the hypothalamus, the pituitary gland, the ovaries and uterus [22]. Its final common pathway is pregnancy or menstruation in absence of pregnancy [23]. Females begin the menstrual cycle at the time of puberty, around age 13 and experience it monthly until around their late forties to middle fifties, except when interrupted by pregnancy. Its interval is between 20-35 days, though the average is 28 days. The normal duration of the menstrual flow is 3-5 days, but flows of 3 days and 8 days can occur in normal women [24]. The amount of blood lost may range

normally from slight spotting to 80 mL; the average amount lost is 30 mL. Loss of more than 80 mL is abnormal [24, 25]. The menstrual cycle can be divided into three phases: the follicular phase, ovulation and the luteal phase [26, 27]. The length of each phase varies from woman to woman and from cycle to cycle [26].

Follicular Phase:

It also called proliferative phase because the lining of the uterus is stimulated to grow, or proliferate, during this time through the influence of a rise in FSH [28]. During the first days of the cycle, a few ovarian follicles are stimulated and competed with each other for dominance [29]. Under the influence of several hormones, then these follicles will stop growing, only one dominant follicle will continue to mature [30], which is called a tertiary or Graafian follicle, from which increasing amounts of estradiol has been secreted. Throughout the entire follicular phase, rising estrogen level in the blood stimulates growth of the endometrium and myometrium of uterus and also causes endometrial cells to up regulate the receptors for progesterone. It has effect on the cervix by producing fertile cervical mucus [24-31].

Ovulation:

Ovulation is the process of releasing secondary oocyte [32]. During the follicular phase, estradiol suppresses production of luteinizing hormone LH [33]. When the ova have nearly matured, it secretes enough estradiol to trigger the acute release of LH [34]. A surge in LH secretion triggers ovulation [35]. It normally occurs 30 (± 2) h after the beginning of the LH surge (when LH is first detectable in urine) [36]. Its release matures the ova and weakens the wall of the follicle in the ovary, causing the fully developed follicle to release its secondary oocyte, which matures into an ootid and then becomes a mature ovum [37]. It has a diameter of about 0.2 mm [38]. After being released into the peritoneal space, the ova are swept into the fallopian tube by the fimbria, which is a fringe of tissue at the end of each fallopian tube [39]. After about a day, an unfertilized ovum will disintegrate or dissolve in the fallopian tube [39, 40].

Luteal Phase

It also called the secretory phase [41]. After ovulation, under the effect of the pituitary hormones FSH and LH, the remaining parts of the dominant follicle changed into corpus luteum, which is the solid body formed in an ovary after the egg has been released into the fallopian tube, which continues to grow for some time after ovulation and produces significant amounts of progesterone and to a lesser extent, estrogen [42]. Progesterone plays a vital role in making the endometrium receptive to implantation of the blastocyst and supportive of the early pregnancy [43]. The endometrium becomes highly vascularized, slightly edematous, under the influence of estrogen and progesterone [44]. The hormones produced by the corpus luteum also suppress production of the FSH and LH [45]. As a result, the levels of FSH and LH fall quickly, and if fertilization does not happen, the corpus luteum degenerates at about 10 days after ovulation and the concentrations of estrogen and progesterone decline markedly [45].

From the time of ovulation until progesterone withdrawal and menstruation starts, the process typically takes about two weeks while, the follicular phase often varies in length from cycle to cycle; and the length of luteal phase will be consistent [48, 49].

Menstrual Abnormalities

The menarche is the first menstruation which is one of the later stages of puberty in girls [50, 51]. The average age of menarche in girls is 12 years, but the normal range is between ages 8 and 16 [52]. Factors such as heredity, diet and overall health can accelerate or delay menarche [53-56]. The absence of ovulation with menstrual periods at fairly regular intervals is called anovulation [57]. Anovulatory cycles are the rule for the first 1 to 2 years after menarche and again before the menopause [58, 59]. Amenorrhea is the absence of menstrual periods [60]. If menstrual bleeding has never occurred, the condition is called

primary amenorrhea [61]. Primary amenorrhoea is the absence of menstruation in a woman by the age of 16 [61, 62]. As pubertal changes precede the first period, or menarche, women by the age of 14 who still have not reached menarche, plus having no sign of secondary sexual characteristics, such as the larche or pubarchethus are without evidence of initiation of puberty are also considered as having primary amenorrhoea [63]. Secondary amenorrhoea is where an established menstruation has ceased for three months in a woman with a history of regular cyclic bleeding, or nine months in a woman with a history of irregular periods [64].

Thalassemia

Historical aspects

In 1925, Thomas Cooley a Detroit pediatrician described severe type of anaemia in children of Italian origin [65]. He noted abundant nucleated RBCs in the peripheral blood, which, as he initially thought, was erythroblasticanaemia which is an entity that Von Jaksh described earlier [6, 66]. Before that, Cooley realized that erythroblastemia is neither specific nor essential in this disorder and that the term erythroblasticanaemia was nothing but a diagnostic catchall. Although Cooley was aware of the genetic nature of the disorder, he failed to investigate the apparently healthy parents of the affected children [66].

Genetic basis:

All types of thalassemia are recessively inherited [67], meaning that a genetic change must be inherited from both parents [68]. Beta-thalassemias are heterogeneous at the molecular level [69]. All individuals have two normal copies of the beta globin gene, which is located on chromosome 11 [69, 70], which makes the beta globin component of normal adult hemoglobin (HbA) [70]. There are approximately 200 genetic mutations that have been described as causing beta thalassemia, designated as either beta 0 (β^0) or beta + (β^+) mutations [71, 72].

β^0 characterized by the complete absence of beta chain production results from deletion, initiation codon nonsense, frameshift, and splicing mutations, especially at the splice-site junction [72, 73]. On the other hand, β^+ thalassemias, characterized by reduced production of the beta chains that are produced by mutations in the promoter area, may be divided into severe, mild, and silent [72-74].

Both affected β-globin genes produce severeanaemia and a potentially life-threatening condition [75]. The severity of the disorder depends in part, on the combination of genes that have been inherited: (β^O /β^O); (β^O /β^+); (β^+ /β^+) [76]. The β^+ thalassaemia genes vary greatly in their ability to produce normal haemoglobin (β^+, β^{++} and β^{+++}); consequently, the clinical picture is more complex than it might otherwise be the case for the three possibilities outlined [75, 76].

The severity of the disease is influenced by the exact thalassemia mutations inheritance as well as other genetic and environmental factors [77].

Geographical distribution of thalassemia:

Beta-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East [78, 79]. The highest carrier frequency is reported in Maldives (18%) Cyprus (14%), Sardinia (10.3%) and Southeast Asia (3%-5%) [80]. The high gene frequency of beta-thalassemia in these regions is most likely related to the selective pressure from Plasmodium falciparum malaria [81].

Alpha-Thalassemia (α):

Alpha-thalassemia is the result of changes in the genes for the alpha globin component of hemoglobin [82]. The mildest form has one alpha globin gene missing [83]. Affected individuals generally have no symptoms, but they can pass on the genetic abnormality to their children so, it is also called silent carrier [84].

Alpha-thalassemia (α-thalassemia) has two clinically significant forms: hemoglobin Bart hydropsfetalis (Hb Bart) syndrome and hemoglobin H

(HbH) disease [85]. Hb Bart syndrome, the more severe form, is characterized by fetal onset of generalized edema, pleural and pericardial effusions, and severe hypochromic anemia, in the absence of ABO or Rh blood group incompatibility [86]. Clinical features include: hepatosplenomegaly, extramedullary erythropoiesis, hydrocephaly, and cardiac and urogenital defects [87]. Death usually occurs in the neonatal period [88]. HbH disease is characterized by microcytic hypochromic hemolytic anemia, hepatosplenomegaly, mild jaundice, and sometimes thalassemia-like bone changes [89]. Carriers of α^0 thalassemia (α-thalassemia trait) show microcytosis, hypochromia, and normal percentages of HbA2 and HbF [90]. Carriers of α^+-thalassemia (α-thalassemia silent carrier) have either a silent hematologic phenotype or present with a moderate thalassemia-like hematologic picture [91]. Homozygosity for α^+-thalassemia results in an α^0-thalassemia (α-thalassemia trait) hematologic phenotype [92].

Beta-thalassemia (β):

1-β-thalassemia major (βTM):

The biochemical signature of β-thalassemia is reduced synthesis of the β-globin subunit of HbA (α2β2) [93]. Individuals inheriting two β-thalassemic alleles experience a profound deficit in β-chain production (homozygous) like in (βTM), and this impairment leads to excess production of α-globin [94]. No compensatory regulating mechanism exists when this occurs. Therefore, in β-thalassemia, the excess of alpha-globin chains forms insoluble tetramers that accumulate and precipitate in the erythroid progenitors, forming inclusion bodies that cause oxidative membrane damage within the RBCs and immature developing erythroblast in the bone marrow. This leads to premature death of many late erythroid progenitors' in bone marrow and spleen [95, 96].

Clinical presentation of thalassemia major occurs between 6 and 24 months [97]. Affected infants fail to thrive and become progressively pale. Feeding problems, diarrhea, irritability, recurrent bouts of fever, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur [97, 98]. In some developing countries where due to the lack of resources patients are untreated or poorly transfused, the clinical picture of thalassemia major is characterized by growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes resulting from expansion of the bone marrow [99]. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes (bossing of the skull, prominent malar eminence, depression of the bridge of the nose, tendency to a mongoloid slant of the eye, and hypertrophy of the maxillae, which tends to expose the upper teeth) [100].

2-β-thalassemia Intermedia

Clinical features are pallor, jaundice, cholelithiasis, liver and spleen enlargement, moderate to severe skeletal changes, legulcers, extramedullary masses of hyperplastic erythroid thrombotic complications resulting from iron accumulation and hypercoagulable state secondary to the lipid membrane composition of the abnormal red blood cells [101].

3-β-thalassemia minor

Carriers of thalassemia minor are usually clinically asymptomatic but sometimes have a mild anemia. When both parents are carriers, there is a 25% risk at each pregnancy of having children with homozygous thalassemia [102].

Pathophysiology of β-thalassemia

The basic defect in β-thalassaemia is a reduced or absent production of β-globin chains with relative excess of α-chains [70]. The direct consequences are a net decrease of the haemoglobin production and an imbalance of the globin chain synthesis [92, 103]. The former is more evident in carriers, leading to a reduction of mean cell haemoglobin and mean cell volume, and has a minor clinical significance [104]. The latter has dramatic effects on the red cell

precursors, ultimately resulting in their extensive premature destruction in the bone marrow and in the extramedullary sites [70, 105, 106].

This process is referred to as "ineffective erythropoiesis" and is thehallmark of β-thalassaemia [107]. Peripheral haemolysis contributing to anaemia is less prominent in thalassaemia major than in thalassaemia intermedia, and occurs when insoluble α globin chains induce membrane damage to the peripheral erythrocytes [107, 108]. The first response to ineffective erythropoiesis and anaemia is an increased production of erythropoietin [109], causing a marked erythroid hyperplasia, which, in turn, may produce skeletal deformities, osteoporosis, and occasionally extramedullary masses, and contributes to splenomegaly [108].

Untreated or undertreated thalassaemia major patients have retarded growth as a result of anaemia and the excessive metabolic burden imposed by erythroid expansion [108, 110]. Anaemia may produce cardiac enlargement and sometimes severe cardiac failure. Ineffective erythropoiesis is also associated with increased iron absorption, which occurs mainly from increased intestinal absorption of iron caused by deficiency of hepcidin, a 25-amino acid peptide produced by hepatocytes that plays a central role in the regulation of iron homeostasis [111, 112].

Haematological Parameters:

Beta-thalassemia comprises a group of different clinical and hematological pictures [113]. The effect of β-thalassemia on haematological parameters is related to the exact phenotype, which depends on the number of genes affected [114]. Thalassemia major is the most severe form of this syndrome and requires frequent blood transfusions [115].

Hemoglobin (Hb)

Hemoglobin is an iron-rich protein in RBCs. It carries oxygen to all parts of the body. It also carries CO_2 (a waste gas) from body to the lungs where it exhaled [116]. People who have thalassemia can have mild or severe anemia, this condition is caused by a lower than normal number of RBCs, or not enough Hb in RBCs [98]. Normal Hb variant also called HbA has four protein chains: 2 α-globin and 2 β-globin chains [117]. The two major types of thalassemia: α- and β- are named after defects in these protein chains [118].

Red Blood Cell Indices and Morphology:

Patients with β-thalassemia show RBC morphologic changes [microcytosis, hypochromia, anisocytosis, poikilocytosis (spiculated tear-drop and elongated cells)], and nucleated RBC (i.e erythroblasts). Typical beta-thalassemia carriers are identified by analysis of RBC indices, which shows microcytosis (low MCV) and reduced content of Hb per red cell (low MCH) [119].

Heterozygous carriers of β-thalassaemia, usually display a low mean cellular haemoglobin (MCH), a low mean cell volume (MCV), and an increased level of HbA_2, which may be associated with low normal or slightly subnormal haemoglobin levels [108, 120]. Peripheral blood smear shows less severe erythrocyte morphologic changes than affected individuals and erythroblasts are normally not seen [121]. β-thalassaemia major is characterized by reduced MCV > 50 and < 70 fl and MCH >12 and < 20 pg. Thalassaemia intermedia is characterized by MCV between 50 and 80 fl and MCH between 16 and 24 picogram (pg) [122]. Affected individuals show microcytosis, hypochromia, anisocytosis, poikilocytosis (spiculated tear-drop and elongated cells), target cells and erythroblasts. The number of erythroblasts (nucleated red blood cell) is related to the degree of anaemia and is markedly increased after splenectomy [108].

Biochemical Findings in β-Thalassemia

Iron

Iron is essential trace element present in almost all cells of the body [123, 124]. Human body requires iron for the synthesis of oxygen carrying protein called haemoglobin found in red blood cells, and myoglobin which is also a protein found in muscles [125]. It also takes part in the production of other

important proteins in the body such as for DNA synthesis and cell division [126]. Iron is transported through the blood by the serum protein, called transferrin [127], which is normally 30% saturated for with iron [128]. The total iron-binding capacity (TIBC) reflects the status of iron in the body and is defined as the amount of iron needed for 100% transferrin saturation [129], when iron is present in excess amounts in the body it will lead to hemochromatosis, which may a be primary or secondary [130]. Secondary hemochromatosis occurs in diseases like thalassemia due to iron overload especially in thalassemia major where repeated blood transfusions are required [131]. Beta thalassemia major patients require frequent blood transfusions which lead to iron overload in the absence of effective chelation therapy [132]. This iron deposits in thalassemic patients can exceed from storage and detoxification capacity of ferritin and also fully saturates transferrin and leads to the formation of free iron which accumulates in blood and tissues [133]. This free iron will cause the formation of very harmful compounds, such as hydroxyl radical (OH). The hydroxyl radicals are highly reactive and attack lipids to form lipid peroxides which contribute to oxidative stress [134, 135].

Serum Ferritin:

Ferritin is the main iron-storage protein in the body [136]. Its synthesis is regulated by quantities of iron by means of the interaction of cytoplasmic proteins bound to the messenger ribonucleic acid [137, 138], currently identified as iron regulatory proteins with specific structures of the mRNA, called iron-responsive elements [139].

Advantages of serum ferritin measurement are: easy to assess, inexpensive, repeating measurements are useful for monitoring chelation therapy and the positive correlation with morbidity and mortality [140]. Still there are some disadvantages such as: indirect measurement of iron burden, fluctuates in response to inflammation, abnormal liver function, metabolic deficiencies and the serial measurement that are required [141, 142]. The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in beta thalassaemia major [143]. The association between serum ferritin and levels of body iron is well established and the test is easy to perform compared with other tests for iron overload. When the serum ferritin level reaches 1,000 ng/L (usually after 10th to 12th transfusion), it is generally taken as the point to initiate iron chelation therapy [144].

Glutathione (GSH):

The tripeptide, GSH is the major thiol antioxidant and redox buffer of the cell [145]. The oxidized form of GSH is glutathione disulphide (GSSG) [146]. GSH is highly abundant in the cytoplasm, nuclei, and mitochondria; it is the major soluble antioxidant in these cell compartments [147]. Because GSH is synthesized in the cytosol by the sequential action of glutamate-cysteine ligase and glutathione synthetase, its mitochondrial presence requires inner membrane transport, two mitochondrial electroneutral antiport carrier proteins have been shown to have the capacity to transport GSH, the dicarboxylate carrier protein and the 2-oxoglutarate carrier protein [148, 149]. GSH in the nucleus maintains the redox state of critical protein sulphydryls that are necessary for DNA repair and expression [150]. Oxidised glutathione is accumulated inside the cells and the ratio of GSH/GSSG is a good measure of oxidative stress of an organism [151].

Hormonal finding in β- thalassemia

Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Estradiol (E2) and Gonadotropin Releasing Hormone (GnRH):

FSH and LH, secreted by the gonadotropin cells of anterior pituitary gland, glycoprotein hormones each with a molecular weight of 30 kilodaltons [152], are required in homeostasis of fertility regulation via the hypothalamic-pituitary-gonadal axis which has been well established in both women and men [153]. FSH

contains two different subunits (α & β) linked by no covalent bounds [154, 155]. The α subunit shares structural homology with LH, while the β subunit is unique [156]. In women, FSH exerts its effect directly on ovarian granulosa cells [157], essential for growth and maturation of ovarian follicles [158], while LH is required for rupturing of Graafian follicle and ovulation [159].

Estradiol is the most potent natural estrogen produced by the Graafian follicle of the ovary and the placenta [160] and in smaller amounts by the adrenals, and the male testes [161]. Target organs for estradiol include the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and skin [162].

Delayed puberty or primary amenorrhea defined as no evidence of pubertal development by the age of 13 year in girls or by the age of 14 years in boys is extremely common in patients with transfusion dependent thalassemia major [163]; children with thalassemia at puberty LH pulsatility index below the mean for pubertal stage compared to normal children [164] and FSH level were normal for pubertal stage in 76% patients [165] and only 12% of the patients had FSH level significantly lower than expected for their stage of puberty and 12% had high FSH levels which suggest early gonadal failure [103]. Basal serum LH and FSH of patients with beta thalassemia were significantly lower compared with those values in normal pubertal controls and did not differ significantly to those found in prepubertal controls [166]; on the other hand, basal serum estradiol concentrations of all thalassemia patients with primary amenorrhea were below the normal values [167].

Ultrasonographic findings in β-thalassemia:

Ultrasonography is the most frequently used imaging investigation in the assessment of the female genital tract [168]. Most often the uterus and ovaries are evaluated with the help of two dimensional transabdominal or endovaginal ultrasonography [169]. It also helps in classification and management of disorders of sexual maturation [171].

Conclusion

In conclusion, B-thalassaemia major is a frequent genetic disorder worldwide and responsible for several maternal, foetal and neonatal complications. Awareness about the various aspects of thalassaemia during pregnancy and also the knowledge about maternal and foetal complications related to thalassaemia are very helpful for health care providers to apply the preventive measures and the necessary interventions. For high-risk couples a clear and documented plan for pregnancy and an advice on contraception are very necessary. The antenatal screening and the accurate genetic prenatal diagnosis are useful to early diagnosis. Overall, a standard management protocol should be implemented in all pregnant women with thalassaemia. These management plans are reviewed in details in previous published studies [97, 100, 119, 163].

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