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Anti Mullerian Hormone in Transfusion Dependent B-Thalassemia and Chronic Idiopathic Thrombocytopenic Purpura

Either Karim El-Adham* Radioisotope Department, Nuclear Research Center, Atomic Energy Authority, Dokki 12311, Giza, Egypt

Kahled Mohamed Salama Faculty of Medicine, Cairo University, Egypt

Abstract: Anti-Mullerian hormone (AMH) is a glycoprotein, a member of the transforming growth factor-B super family. This hormone is a sensitive marker of ovarian reserve. The present study aims to measure the Anti-Mullerian hormone in thalassemic females receiving the regular blood transfusion as well as patients of chronic idiopathic thrombocgtopenic purpura and age and sex matched controls. Serum Anti-Mullerian hormone was measured by ELISA and Ferritin were measured by RIA. Clinical evaluation was done for all patients including anthropometric measurements, pubertal staging and history taking. Results of the study were analyzed by appropriate statistical methods. Obtained results revealed that the values of Body Mass Index as well as Anti-Mullerian were significantly higher in controls than thalassemics and chronic idiopathic thrombocytopenic purpura and there was a negative correlation between serum Ferritin and Anti-Mullerian hormone. Moreover, Anti-Mullerian hormone was significantly higher in patients receiving Desferal than in those receiving Deferriprone. Reduced Anti-Mullerian hormone in thalassemics as well as chronic ITP patients are considered an important indicator declines in ovarian function which entail modification in the therapeutic plans for thalassemic and chronic ITP patients.

Keywords: Anti-Mullerian hormone thalassemia; ITP.

1. Introduction

B-thalassemia is a worldwide distributed hereditary disease. It is caused by a genetic deficiency in the formation of B-globin chains. It causes a life-threatening microcytic anemia [1].

In thalassemia major anemia results from intravascular hemolysis, early destruction of the RBCS and insufficient erythropoiesis. Clinical manifestations always start at an early age with severe anemia, hepatosplenomegaly, heart failure and bone deformations [2]. Blood transfusion is the recommended treatment. However, repeated transfusions lead to iron overload and iron deposition in various organs like liver and heart leading to tissue damage and organ dysfunction. Iron deposition in the pituitary gland and pancreas lead to endocrinopathies, diabetes and growth failure [3].

Despite the great advance in chelation therapy still iron overload is the important cause of morbidity and mortality in thalassemics [4].

Immune thrombocytopenic purpura (ITP) is an immune mediated disorder in which platelets are surrounded by auto-reactive antibodies and destructed by the reticuloendothelial system. The clinical features of ITP are varying from a case to another as well as the etiology. ITP may be preceded by upper respiratory tract infection, gastroenteritis, flu or immunization. Also, ITP may have an insidious onset and a chronic course symptoms and signs vary from mild bruising, mucosal bleeding or frank hemorrhage which may be life-threatening as intracranial hemorrhage [5].

A homodimeric glycoprotcin, Anti mullerian hormone, is a member of transforming growth factor B super-family. Several studies about AMH were done over the last few years. AMH levels are a sensitive reflection of the ovarian follicular reserve and they are the most sensitive markers of ovarian aging and early ovarian failure [6]. It was documented that females with lower AMH have lower antral follicular count and produce a significantly lower number of oocytes compared with females of higher number [7]. This study aims to demonstrate the AMH in pubertal transfusion-dependent patients with thalassemia major and pubertal patients with ITP as the reproductive function in patients with different hematological conditions in an important issue.

*Corresponding Author

2. Subjects and Methods

The study included 50 females of ages between 10-15 years old and classified into three groups.

Group I: Consists of 20 transfusion dependent thalassemia major patients.

Group II: Consists of 10 patients suffering from chronic ITP.

Group III: Consists of 20 healthy age marching females as a control group.

All cases were selected from the hematology clinic, New Children Hospital, Cairo University.

2.1. All groups were subjected to

a) Clinical examination

- Anthropometric measurements, including weight, height, body mass index (BMI).
- Pubertal staging: A standard system for staging of secondary sexual characters is used known as Tanner staging.

It shows a score for the normal pubertal development in the size of female breast, female pubic hair and female axillary hair. All participants of the study achieved normal puberty.

b) History

i) For thalassemia

- Onset, course, duration of the disease.
- Consangiuly.
- Frequency of blood transfusion.
- Intake of iron chelation therapy including Desferal of Deferriprone.

ii) For chronic ITP

- Onset, course, duration of disease.
- Steroid intake.
- Blood transfusion.

3. Biochemical Analysis

3.1. Determination of Ferritin

Ferritin level was assayed by immunoradiometric assay (IRMA) kit (Immunotech s.r.o). The immunoradiometric assay of ferritin is a "sandwish" type assay. Mouse monoclonal antibodies directed against two different competition is done epitopes of ferritin molecule and so on. The samplers are incubated in tubes coated with the first monoclonal antibody and the second monoclonal antibody labeled with iodine-125. After incubation, the content of tubes is aspirated and the tubes are rinsed so as to remove unbound iodine-125 labeled antibody. The bound radioactivity is then determined in a gamma counter. The ferritin concentrations in the samples are obtained from the standard curve. The level of ferritin in the samples is directly proportional to the radioactivity.

3.2. Determination of Anti Muellerian Hormone

Anti-Muellerian hormone (AHM), Muellerian-inhibiting factor or Muellerian-inhibiting substance (MIS) level was assayed by Enzyme-linked immunosorbent assay (ELISA) kit (ElAab, Catalog No: E0228h, China). The Microtiter plate provided in this kit has been pre-coated with an antibody specific to Muellerian-inhibiting factor. Standards or samples are then added to the appropriate Microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for Muellerian-inhibiting factor and Avidin conjugated to Horseradish Peroxidase (HRP) was added to each micro plate well and incubated. Then, a TMB substrate solution was added to each well. Only those wells that contain Muellerian-inhibiting factor, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450±2 nm. The concentration of Muellerian-inhibiting factor in the samples was then determined by comparing the O.D. of the samples to the standard curve.

FSH and LH hormone levels were evaluated by Immunoenzymetic assay by ELISA Reader with the standard kits [8].

4. Statistical Analysis

Obtained data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing two groups when normally distributed and Mann Whitney *U* test for independent samples when not normally distributed. Comparison of numerical variables between more than two groups was done using one-way analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons in the normal data and Kruskal Wallis test with posthoc multiple 2-group comparisons in non-normal data. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-normal

variables. *P* values less than 0.05 were considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

5. Results

Table 1 shows the baseline characteristics of the study groups concerning age, weight, and height with no significant difference between all groups and reported the mean of serum Ferritin in thalassemia.

Table-1. Baseline characteristics of the study groups

Group	Control (n=20)	Thalassemia (n=20)	ITP (n=10)
Age (years)	14.2 ± 0.77	14.2 ± 0.99	13.7 ± 0.67
Height (cm)	148.3 ± 2.17	136.0 ± 12.36	137.4 ± 4.22
Weight (kg)	47.1 ± 2.63	32.4 ± 9.08	44.2 ± 2.04
Serum ferritin (mg/dl)		1912 ± 885	

Data are described as mean ± SD

Table 2 shows a comparison of body mass index and anti-Mullerian hormone among all groups. It stated that BMI and anti Mullerian hormone are significantly higher in controls than both thalassemias and ITP with a P<0.001. A comparison of FSH, LH between the studied groups was found without significant difference.

Table-2. Comparison of body mass index (BMI) and anti-Mullerian hormone (AMH), FSH, LH between the groups of study

Group	Control (n=20)	Thalassemia (n=20)	ITP (n=10)	P value
BMI (kg/m^2)*	21.4 ± 1.07	17.2 ± 2.20	23.5 ± 1.48	< 0.001
AMH (ng/ml)†	46.2 ± 3.2	0.5 ± 0.08	0.5 ± 0.02	< 0.001
LH (mIu/ml)	5.8 ± 0.88	4.9 ± 1.2	5.2 ± .76	0.007
FSH (mIu/ml)	7.4 ± 1.1	6.6 ± 1.3	6.4 ±0.93	0.02

Data is reported as mean ± SD;
*: all groups are significantly different from each other ($p < 0.01$)
†: both groups are significantly different from control ($p < 0.001$)

Table 3 shows a comparison between serum Ferritin and anti-Mullerian hormone among patients receiving desferal (n = 7) and those receiving Deferriprone (n = 7) with no significant difference.

Table-3. Comparison of ferritin and anti-Mullerian hormone (AMH) between different treatment drugs among thalassemia group

Group	Desferal (n=7)	Deferriprone (n=7)	P value
Serum ferritin (mg/dl)	2073 ±970	2317 ±666	0.593
AMH (ng/ml)	0.545 ±0.040	0.473 ±0.066	0.030

Data is reported as mean ± SD

Fig (1) shows an inverse relation between serum Ferritin and AMH among thalassemics cases (P<0.001).

Figure-1. Correlation between ferritin and AMH among thalassemiac cases ($p < 0.001$)

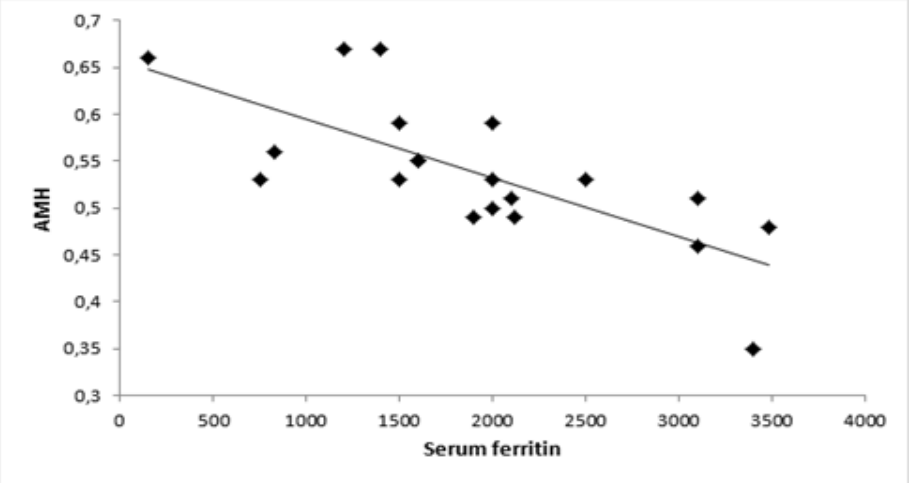


Fig (2) shows mean serum anti Mullerian hormone (AMH) between the controls, thalassemics and ITP.

Figure-2. Mean serum anti-Mullerian hormone (AMH) between the three study groups (p < 0.0)

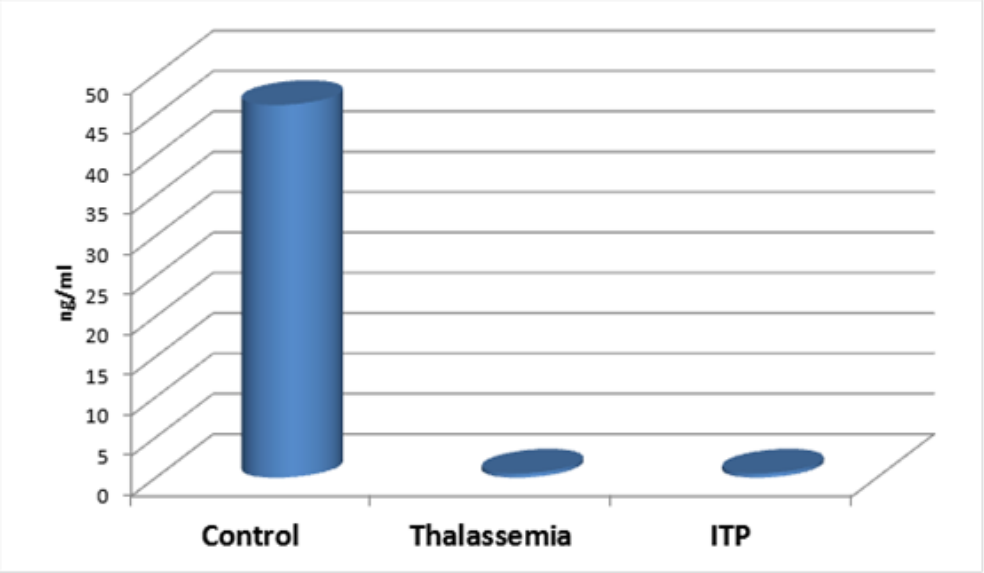
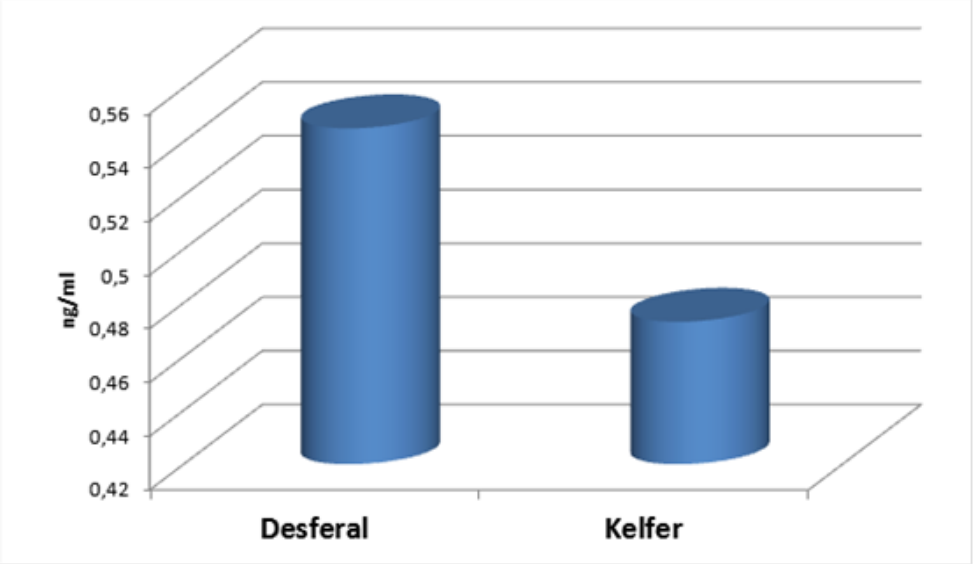


Fig (3) Shows mean serum anti Muellerian hormone (AMH) between thalassemics receiving Desferal and those receiving Deferriprone, it was higher in patient using Desferal than those using Deferriprone with a P<0.030.

Figure-3. Mean anti-Mullerian hormone (AMH) between thalassemia cases on different therapeutic modalities (p = 0.030)



6. Discussion

Thalassemia is one of the most serious health problems, especially in the developing countries. Recently the survival of thalassemics increases and the prevalence of complications due to iron deposition in tissues and other morbidities also increases [9].

Immune thrombocytopenia (ITP) constitute the prototype of autoimmune disorder characterized by thrombocytopenia resulted from antibody mediated platelets destruction and these antibodies may also cause impaired platelets production by damaging megakaryocytes or blocking their function to release platelets. This study aims to evaluate the integrity of ovarian function and the ovarian reserve in both ITP and thalassemia major.

It was demonstrated that serum AMH was significant lower in both thalassemics and ITP patient than age matched controls with a P value <0.001. Thalassemia serum ferritin was inversely proportional to serum AMH. These results are close to the previously reported that AMH was significantly higher in controls than thalassemias and that there was an inverse correlation between AMH and Ferritin [10]. It was reported that ferritin is slightly higher in patients using desferal than those using Deferriprone with no difference in AMH between patients on different chelation modalities. The poor ovarian reserve in thalassemics may be due to iron overload which results from repeated transfusions. Iron toxicity to the anterior pituitary causes hypogonadism due to decreased production of FSH and LH.

Decreased gonadotropins may play a role in the failure of maturation of the ovaries and the production of transferrin bound iron (NTBI) may play a role in the damage of the reproductive tissue and decreased ovarian reserve, especially that some studies reported decreased volume of the ovary and antral follicular count in thalassemic females in comparison to normal controls [11].

Several complications caused by iron overload may be due to the presence of different genotypes of B globin

gene mutations (B° or B?). Different genotypes also result in different amounts of transfusions and different degree of autoimmunity and vulnerability to radical damage [12].

In 2011 a study was done by Sylvia, et al to investigate the reproductive capacity in iron overloaded women with thalassemia, major similarity they attributed the decreased follicular count and ovarian volume to the decreased gonadotropin secretions (LH, FSH, Intribin B and estradiol).

There was no decrease in the AMH levels and this suggests that the ovarian reserve in the patients of that study was mostly normal, yet, this can represent only early and prenatal follicles which aren't affected by the decreased gonadotropin secretion There was an inverse correlation reported between AMH and NTBI suggesting that iron overload plays a role in decreased reproductive capacity in thalassemic females Sylvia, *et al.* [13]. Ovarian tissue damage may occur due to direct toxicity of ovarian iron deposition or an increase in the reactive oxygen spears and decreased enzymatic antioxidant activity which act as the defense mechanism to tissue damage leading to premature aging [14] Most studies concluded that in thalassemic females reproductive capacity decreases with age and that there is on inverse correlation between AMH and age [13] there may be an association between high cardiac iron and other endocrinopathies occurring secondary to iron overload especially with the long duration of blood transfusion.

The association of cardiac iron with NTBI suggests that labile iron causes myocardial damage and ovarian tissue damage, Free iron may be potentially toxic and it may have a major role in impaired reproductive capacity in thalassemia [15] In our study there was serum ferritin wasn't significantly higher in patients using Deferriprone than those using Deferriprone, but AMH was significantly lower in patients on Deferriprone than those on Desferal also Deferriprone in that study has a better compliance than Desferal. As for the Egyptian experience in chelation therapy several investigators reported that beta- thalassemia major patients with transfusion iron overload can be effectively treated with an alternative therapy of DFO/DFP, this leads to decreased mean serum ferritin, and that deferriprone a small molecule that permeates all tissues may be more effective in removing cardiac iron and similarly ovarian iron, improving cardiac and ovarian function. In 2013 a study was done by Paraskevi et al about the Beta thalassemia major patients and female fertility concerning the role of iron and iron induced oxidative stress.

The study proved also that impaired fertility in thalassemic females is due to hypogonadotropic hypogonadism and this may be caused by to iron deposition and oxidative stress causing several endocrine disturbances, and causing impaired metabolism of hormones and serum antioxidants. The results are close to those produced previously [13].

In our study it was reported that serum AMH was significantly higher in controls than ITP, yet there wasn't a between ITP patients and thalassemics. This may be attributed to the fact that autoimmunity plays an important role in the etiology and pathophysiology of ITP [16]. Several studies reported that thrombocytopenia results not only from antibody mediated platelet destruction, but also it could be the same antibodies that mediate platelet destruction also mediate impaired platelets production by damaging mega korocytes or blocking their ability to release pro platelets [17] auto antibodies mediate tissue damage and similarly CAN cause ovarian tissue injury or decreased production of oocytes.

ITP is also a common comorbidity with other autoimmune diseases as SLE and rheumatoid arthritis [18]. The first line of therapy for ITP includes corticosteroids, sometimes in combination with IVIG or anti Rh (D). The second line of therapy for ITP may include splenectomy, Rituximab or thrombopoietin receptor antagonists.

The main target of the therapy is to provide a chance for long-term remission [19], perhaps the decreased ovarian reserve and reproductive capacity and may be due to the side-effects of any of the medications used. Also, that there may be an association between autoimmunity and other reproductive problems as endometriosis [20].

Finally impaired fertility and decreased ovarian reserve in thalassemia are due to excessive iron deposition, oxidative distress and overproduction of free radicals leading to ovarian tissue injury and decreased ovarian reserve. Advances in chelation therapy, intake of appropriate anti-oxidants and essential trace elements can improve the extent of tissue damage and the reproductive function.

Further studies are still needed concerning reproduction in both ITP and thalassemia.

7. Conclusion and Recommendations

It could be concluded from this study that AMH is significantly higher in controls than both thalassemics and ITP patient and that AMH is inversely proportional to serum ferritin and that AMH is significantly higher in thalassemics using Desferal than those using Deferriprone. Both of thalassemics and ITP patients suffer from impaired fertility and poor ovarian reserve.

Ovarian function and fertility in both thalassemia and ITP need further investigations to improve life quality in these patients.

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