ORIGIN AL ARTICLE

Study of Comparison of Serum Interleukin-1 Beta Levels in B Thalassemia Major Patients with Normal and Impaired Glucose Tolerance

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ABSTRACT

Background: Beta-Thalassaemia major (βTM) is hemolytic congenital disorder leading to hypochromic microcytic anemia. Due to repeated blood transfusion, patients with βTM develop iron overload and deposition in various body organs which may result in destruction of pancreatic β cells initiating oxidative stress and hypoinsulinemia and eventually result in Type 1 Diabetes Mellitus. Immune-competent cells like macrophages release pro-inflammatory cytokines such as interleukin-1 beta which may be an important clinical indicator of ongoing pancreatic β cell destruction in pre-diabetes phase.
Aim: To evaluate and compare serum interleukin-1β levels in Beta-Thalassemia major children having normal and impaired glucose tolerance.
Methods: 90 patients of Beta thalassemia major aged ≥ 10 years belonging to both genders were taken for study purpose. These patients were divided into 2 study groups. Group A included 45 patients having normal fasting blood glucose and glucose tolerance. Group B included 45 patients with impaired fasting blood glucose levels and glucose tolerance. The study data was then analyzed by SPSS version 21.
Results: The mean age was 11.56±1.06 years and 11.56±1.06 years in group A and B respectivley. Mean BMI was 22.41±1.76 Kg/m^2 and 22.21±2.15 Kg/m^2 in group A and B respectively.With regards to gender distribution, group A comprised of 29 (64.5 %) males and 16 (35.5%) females, whereas in group B, there were 27(60%) males and 18(40%) females. Mean serum ferritin levels were 4428.04±1776.89 ng/ml and 5246.09±3073.06 ng/ml in group A and B respectively. Mean fasting blood sugar was 95.2±9.1 mg/dl and 119.13±3.92 mg/dl in group A and B respectively. Mean oral glucose tolerance was 128.2± 6.94 mg/dl and 166.51±12.56 mg/dl in group A and B respectively. Mean serum interleukin-1β (IL-1β) levels were 2.91±1.30 and 5.24±2.16 pg/ml in group A and B respectively.
Conclusion: Higher interleukin-1 beta levels contribute significantly to development and progression of impaired glucose tolerance in beta thalassemia major patients and hence pose a threat for Type 1 diabetes mellitus progress in these patients.
Keywords: Interleukin-1 beta, Beta thalassemia major, Impaired glucose tolerance

INTRODUCTION

Thalassemia is a frequently prevalent single gene disorder having > 200 different gene mutations causing reduced formation of globulin chains. Thalassemia has two common types: α and β thalassemia due to deficient alpha and beta hemoglobin chains respectively[1].

Beta-thalassemia ranges from asymptomatic thalassemia minor to a milder anemic variety of β Thalssemia intermedia to a grave transfusion depending β thalassemia major. βTM patients are subjected to repeated blood transfusion entire life. Endocrine abnormalities, thyroid disorders, growth retardation, kidney and liver failure are the most common complications in these patients due to iron overload. This creates an excessive pool of free radicals which causes oxidative damage to many cells and tissues[2].

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The reported number of beta thalassemia major patients in Pakistan is around 100,000. Moreover the silent variety β thalassemia minor is prevalent in 8-10 million Pakistani population[3]. Suppression of hepcidin (a key regulator of iron entry in blood) leads to unbalanced iron build up in the body in thalassemia patients[4]. Lifelong blood transfusions and iron chelation therapy are main treatment options along with supportive measures for βTM patients to maintain hemoglobin level above 9g/dl. Splenectomy is opted when required but superlative treatment option is Allogeneic Hematopoietic Transplantation of stem cells[5].

A positive correlation has been reported between iron deposition and damage to pancreatic β cells and oxidative stress in β-Thalassemia major patients, leading to hypoinsulinemia, hyperglycemic state and pre-diabetes[6]. Oversaturation of plasma transferrin leads to more unbound free iron, which is highly reactive and can initiate cytotoxicity through the increased production of reactive oxygen species[7].

Reduced insulin synthesis due to chronic iron toxicity leads to an abnormal glucose tolerance and progression towards full blown diabetes mellitus (DM). Another possible mechanism is immune system activation against pancreatic β cells in βTM patients[8]. The buildup of free iron leads to damage to various biomolecules, cellular membranes and proteins ultimately causing oxidative stress and DNA mutation[9]. Chronic low-grade inflammation mediated by oxidative stress and disturbed immune mechanisms is associated with initiation and progression of DM[10].

In chronic inflammation there is tissue destruction due to cytokine production by various immune cells. It has been reported that DM can also be a manifestation of chronic inflammatory process[11]. Type-1 diabetes mellitus (T1DM) develops due to gradual destruction of pancreatic β cells by body immune system. Genetic predisposition and environmental factors play important role in it. This autoimmunity induced damage to pancreatic beta cells can involve T cells, monocytes and macrophages by producing more pro inflammatory cytokines[12]

Interleukin-1β is an important pro-inflammatory type of cytokine. Immune cells like T cells and macrophages once infiltrate into islet pancreatic cells, produce this cytokine and lead to pathogenesis of T1DM. Interleukin-1β (IL-1β) stimulates reactive oxygen species and nitric oxide synthase enzyme production. This leads to formation of free radical Nitric Oxide which causes cytotoxicity[13]. By combating these inflammatory cytokines, initiation and progression of T1DM can be halted[14].

This study was done to evaluate the possible role of IL-1β in the pathogenesis of T1DM in β-thalassemia major children. Comparison of serum IL-1β levels was done in β-thalassemia major children having normal and impaired glucose tolerance.

MATERIAL AND METHODS

This cross-sectional study was conducted in Post-graduate Medical Institute, Lahore, Pakistan and Thalassemia Center Fatima Jinnah Medical University/ Sir Ganga Ram Hospital, Lahore from 1st December 2017- 31st May 2018 after approval from the Institutional ethical Boards. Total 90 patients divided into two groups A and B (45 patients each group) were included in our study. Non Probability Purposive sampling technique was used.

Group A: Thalssemic patients with normal levels of FBS (fasting blood sugar) i.e., < 110 mg/dl and with normal oral-glucose tolerance i.e., serum glucose level < 140 mg/dl, 2 hours post ingestion 75 gm glucose load[15].

Group B: Thalassemic patients with impaired FBS i.e., 110 - 125 mg/dl and/or impaired oral glucose tolerance i.e., serum glucose level, 140 - 200 mg/dl, 2 hours post ingestion 75 gm glucose load[15].

Inclusion criteria:

- β Thalassemia major (β TM) patients of both genders
- βTM children age ≥ 10 years having multiple blood transfusion
- βTM children with confirmed iron-overload having serum ferritin levels > 2000 ng/ml

Exclusion criteria: Patients having any autoimmune disease which can elevate serum IL-1β level e.g., arthritis, systemic lupus erythematosus, atherosclerosis. Confounders like obesity or metabolic syndrome with insulin resistance

Statistical analysis of data: The statistical analysis of the data was done by using Statistical Package for the Social Sciences (SPSS) version 21 for windows (SPSS Inc., USA). Descriptive results were expressed as mean ± SD (standard deviation). An independent samples t-test was used for comparison of means of variables. P values < 0.05 were taken statistically significant.

Haematological and biochemical analysis: Blood glucose was measured by glucose oxidase method by using Pointe Scientific, INC. kit, USA. Serum ferritin was measured by ELISA method by the help of Biocheck INC. kit, USA.Serum interleukin-1 β was measured by ELISA method by using Glory Science Co. Limited. kit USA.

RESULTS

Fasting blood sugar, oral glucose tolerance, serum ferritin and serum interleukin-1beta were measured in all 90 children of βeta- Thalassemia major. The patients were divided into two balanced groups. Group A consists of 45 patients with normal glucose tolerance labeled if Fasting Blood Sugar FBS < 110 mg/dl and Oral Glucose Tolerance OGT < 140. Group B also comprised of 45 patients with impaired glucose tolerance labeled if FBS = 110 - 125 mg/dl and OGT ≥ 140 and < 200 mg/dl.

The mean age was 11.56±1.06 years and 11.56±1.06 years in group A and B respectively. Mean Body Mass Index (BMI) was 22.41±1.76 Kg/m^2 and 22.21±2.15 Kg/m^2 in group A and B respectivley. Independent sample`t` test did not give us significant difference with regards to age and BMI between two study groups. P-value > 0.05 indicates statistical difference is not significant.

Gender distribution in group A shows that , there were 29 (64.5 %) males and 16(35.5%) females. Group B comprises 27 (60%) males and 18 (40 %) females. The overall mean percentage of males in both groups was 62.25% (56 cases out of 90) and females were 37.75 % (34 cases out of 90) (Table 1).

The serum ferritin levels were 4428.04±1776.89 ng/ml and 5246.09±3073.06 ng/ml in group A and B respectively. Student `t` test shows non significant distribution of serum ferritin levels between the two groups, the p-value being > 0.05. Mean fasting serum glucose was 95.2±9.1 mg/dl and 119.13±3.92 and mean oral glucose tolerance was 128.2±6.94 mg/dl and 166.51±12.56 mg/dl in group A and B respectively. For both of these biochemical investigations, the independent samples `t` test shows statistically, a highly significant difference between group A and group B, p-value was noted as < 0.001. The mean serum interleukin-1βeta (IL-1β) levels were 2.91±1.30 pg/ml and 5.24±2.16 pg/ml in A and B group respectively. When applied the independent samples `t` test shows statistically a highly significant difference in IL-1β levels between A and B group. The p-value was noted as < 0.001 (Table 2).

The mean serum interleukin-1β levels were 2.88±1.25 pg/ml and 5.60± 2.25 pg/ml in group A and B males with a p-Value noted as < 0.001 by the independent samples `t` test which shows marked significant difference statistically in group A and B males. The serum interleukin-1βeta levels

were 2.96±1.42 pg/ml and 4.70±1.94 pg/ml in group A and B females. The p-value was observed as < 0.001 revealing significant difference of serum interleukin-1βeta levels between female children of group A and B (Table 3).

Table 1: Independent sample t test comparing Age, BMI and Gender

Variables	**Group A (n=45)** (mean±SD)	**Group B (n=45)** (mean±SD)	**P value**
Age (Yrs)	11.56 ± 1.06	11.56 ± 1.06	> 0.05
BMI (Kg/m^2)	22.41 ± 1.76	22.21 ± 2.15	> 0.05
Male	29 (64.5 %)	27 (60.0 %)	
Female	16 (35.5 %)	8 (40.0 %)	--

Table 2: Independent samples t test comparing serum ferritin, serum fasting blood sugar, oral glucose tolerance and serum interleukin 1 β levels

Marker	**Group A** (mean±SD)	**Group B** (mean±SD	**P value**
Serum ferritin (ng/ml)	4428.04±1776.89	5246.09± 3073.06	> 0.05
Fasting blood sugar (mg/dl)	95.2 ± 9.1	119.13 ± 3.92	<0.001
OGT (mg/dl)	128.2 ± 6.94	166.51± 12.56	<0.001
Serum IL-1βeta (pg/ml)	2.91 ± 1.30	5.24 ± 2.16	<0 .001

Fig. 1: Comparison of blood glucose fasting (FBS), oral glucose tolerance test (OGT) and serum Interleukin-1β levels, between group A and B

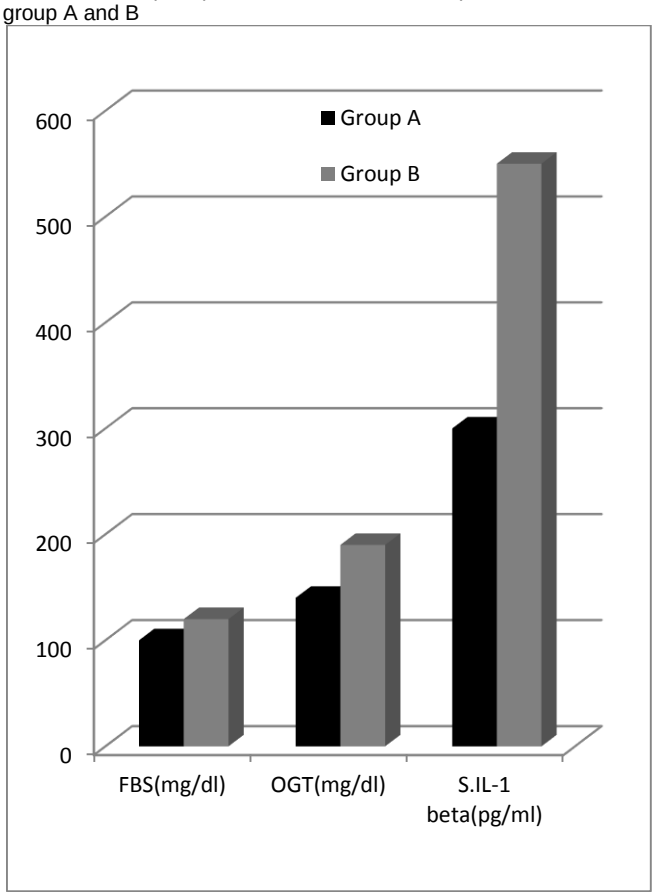

Table 3: Independent samples t test showing comparison between serum interleukin-1β levels in groups A and B gender-wise

Marker(pg/ml)	**Group A** (mean ± SD)	**Group B** (mean ± SD)	**P-value**
Serum Interleukin -1β in males	2.88 ±1.25	5.60 ±2.25	< 0.001
Serum Interleukin-1 β in females	2.96 ± 1.42	4.70 ± 1.94	< 0.001

DISCUSSION

Beta thalassemia is widely prevalent congenital hemolytic disorder. Regular blood transfusions are required to keep life going in such children[16]. Transfusion induced iron overload can lead to development of DM due to secondary hemochromatosis in thalassemia major patients[17]. Endocrinopathies and metabolic disorders like Diabetes Mellitus are the major complications associated with β thalassemia major. The prevention of such comorbidities is an important target to be achieved[18]. Many important cytokines such as interleukin-1 beta (IL-1β) are considered important inflammatory mediator causing various organs damage[19].

Our study was done to compare serum levels of interleukin-1 beta in β Thalassemia major children with normal and impaired blood glucose levels as evaluated by oral glucose tolerance test. For this study, 90 patients were divided into 2 groups with 45 patients in each group. Group A comprises of beta thalassemia major patients with normal glucose levels and normal oral glucose tolerance and group B patients have impaired serum fasting glucose levels and impaired oral glucose tolerance.

Statistical evaluation revealed a statistically significant difference between both groups with regards to serum interleukin-1 beta levels. Beta Thalassemia major patients having impaired levels of blood glucose displayed marked elevation of serum IL-1 beta levels in comparison with beta thalassemia patients with normal blood glucose levels. Same was the observation recorded when the IL1- beta levels were compared with regards to gender, in both groups.

In Group A children ,the serum ferritin levels (in mean±SD) were 4428.04±1776.89 ng/ml. On the other hand Group B serum ferritin values were 5246.09±3073.06 ng/ml. Independent sample `t` test describes non-significant distribution of serum ferritin levels between the two groups, the p-value is > 0.05.

In a study done in 2018 by Ansari *et al*, the mean ferritin serum levels were reported as 5156 ng/ml, which is in agreement with our research[20]. Pennell *et al* in 2015 reported that the median ferritin serum level (range) was noted as 4902 (613 – 13,104) ng/ml and 5896 (2368 – 9898) ng/ml in both study groups, which is in line with our study[21].

According to our study groups,there are 50% beta thalassemic patients having normal serum sugar and normal glucose tolerance status and 50 % patients have impaired fasting serum sugar and impaired oral glucose tolerance status.

Tehseen *et al* in 2017, Azami *et al* in 2017 and Rizvi *et al* in 2017 described that the percentage frequency of β

Thalassemia major children having impaired oral glucose tolerance was 24.23%, 9.6% and 10.56% respectively[22,23,24]. Variation in these percentages of patients with impaired tolerance of glucose was because of random sampling.

Serum interleukin-1 β levels in both groups were as follows: Group A shows 2.91±1.30 pg/ml and group B shows 5.24±2.16 pg/ml value in mean±SD. The p-value was found to be < 0.001and independent samples `t` test reveals marked statistical difference in interlekin-1 beta levels in both comparison groups. Our study shows marked increase in interleukin-1 beta levels in beta thalassemic patients having impaired serum fasting sugar and impaired glucose tolerance. In future this interleukin-1 beta cytokine can be potential therapeutic target to stop initiation and progression of DM in thalassmic patients.

Limited data is available which shows comparison of this biomarker regarding oral glucose tolerance in same beta thalassmic population. Perez *et al* in 2004 reported the levels of serum IL-1β as 9.3±7.3 pg/ml in freshly diagnosed DM patients in comparison with controls value of 4.9±3.8 pg/ml[25]. Dogan *et al* in 2006 reported IL-1β serum levels as 13.7±2.3 pg/ml in recently diagnosed DM patients in contrast to the value of 3.8±0.9 pg/ml in controls[26]. Netea *et al* in 1997 reported IL-1β levels in serum as 5 – 13.5 pg/ml in freshly diagnosed DM patients[27]. El-Nawawy *et al* in 1998 described serum IL-1β values of 31.8±7.7 pg/ml in diabetic patients and values of 21.2 + 6.4 pg/ml were found in healthy controls[28]. Younis *et al* in 2017 mentioned serum Interleukin-1β levels as 37.5±24 pg/ml in DM patients with drug metformin as only therapy but reduces to 34.5±11 pg/ml when another drug Vildagliptin is also added to Metformin with marked drop of HbA1c levels[29].

The values depicted here are not in perfect match with our values but the values are increased in the patient population to an extent, as seen in our study.

Further large scale studies are needed to elaborate the fine details of the complex inflammatory phenomenon of DM progression in beta thalassmia patients

Recommendations: With this study we came to a conclusion that serum levels of interleukin-1 beta are enhanced in beta Thalassemia major children having impaired oral glucose tolerance. So it is strongly recommended that all beta thalassemic patients should be screened for impaired glucose metabolism and interleukin-1 beta levels to stop development and progression of full blown Diabetes Mellitus due to oxidative stress and inflammatory process.

CONCLUSION

Higher interleukin-1 beta levels contribute significantly to development and progression of impaired glucose tolerance in beta thalassemia major patients and hence pose a threat for Type 1 diabetes mellitus progress in these patients.

Limitations of the study: Our study has several limitations:

1. Firslty the number of patients was relatvely small
2. Secondly, the age range was due to limited resources of the study.
3. Further large scale studies are needed to elaborate the fine details of the complex phenomenon of DM progrssion in beta thalassmia patients

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