Objective: To determine the effects of iron overload on Height, Body Mass Index (BMI), Hemoglobin and Serum Ferritin levels in beta thalassemia major patients undergoing regular blood transfusion.

Study Design: Case control study.

Place and Duration of Study: It was carried out at Quaid-e-Azam University, Islamabad in collaboration with Jamila Sultan Foundation Rawalpindi, Thalassemia House Rawalpindi and Pakistan Institute of Medical Sciences (PIMS), Islamabad from 5th January 2010 to 5th December 2014.

Materials and Methods: Total 300 individuals were included in the study out of which 200 were Beta thalassemia major patients and 100 were controlled matched for age and gender with the thalassemic group. They were further divided into 4 groups of <13 years female, ≥13 years female, <13 years male and ≥13 years male (each having 50 thalassemic and 25 control). Height, BMI, Hemoglobin and serum Ferritin levels were determined. Non parametric (Spearman) co-relation co efficient was used to find the correlation between BMI and Ferritin and Hb levels. Data was analyzed through Graph Pad Prism 5.01. P<0.05 was considered statistically significant.

Results: All groups had reduced Height, BMI, Hb and high Ferritin levels as compared to the control groups. Significantly positive (P<0.001) correlation of BMI with Hemoglobin and serum Ferritin levels were observed in thalassemic females of ≥13. While <13 years thalassemic males had significant (P<0.01) negative correlation of BMI with Hemoglobin.

Conclusion: Our study revealed that beta thalassemic patients had reduced height and BMI, associated with high levels of serum ferritin and low hemoglobin.

Key Words: Body Mass Index, Ferritin, Height, Hemoglobin, Thalassemia Major.

Introduction
Thalassaemia major is a hereditary hemolytic disorder which is treated with repeated blood transfusions. About 240 million beta thalassemia carriers are present all over the world. Every year about 100,000 children are born with the disease of thalassemia. On diagnosis of a child with thalassemia homozygous there is a lifelong sequence of blood transfusion every three weeks along with chelation therapy and facing complications due to iron overload and transfusion transmitted infections. These transfusions maintain a hemoglobin level higher than 9.5 gm/dl as anemia effects the normal growth and development of these patients. Despite the fact that blood transfusions are mandatory for the treatment of patients suffering from anemia, repeated transfusions lead to iron overload as human beings do not have the ability to remove the extra accumulated iron. Increased intestinal absorption of iron further worsens the condition, due to iron overload. The iron gets deposited in various organs like liver, heart and endocrine glands which lead to various types of endocrinopathies like hypogonadism and diabetes mellitus which lead to retarded pubertal development in thalassemia major patients. Underweight and under-nutrition may lead to loss of energy and susceptibility to injury and infection, under-function of multiple endocrine systems, as well as distorted body image and other psychological problems. There is increased prevalence of bone disease in patients suffering from thalassemia major as compared to normal individual. The bone growth depends on the sex steroids which regulate...
bone maturity. Thalassemia major patients suffer from hypogonadism and fail to achieve their peak bone mass due to the bone disease which develops during the course of their disease. Serum Ferritin levels above 1000 ng/mL are considered as an iron overload. The levels of serum Ferritin vary among patients getting multiple transfusions. However, the cutting level at which iron toxicity and organ damage takes place is still not identified.

The biochemical screening such as serum Ferritin and Hemoglobin levels are of paramount importance in all beta thalassemia patients in pediatric and adolescent age groups. These levels should be detected and treated for preventing pubertal delay in such individuals which has not been recognized in our part of the world in view of their pubertal growth. Therefore, present study was done to determine the effects of iron overload on Height (cm), BMI (Kg/m2), serum Ferritin (ng/mL) and Hemoglobin (gm/dl) levels along with exploration of the correlation of BMI with serum Ferritin (ng/mL) and Hemoglobin (gm/dl) levels of beta thalassemic patients of pubertal age group undergoing repeated blood transfusions with chelation therapy.

Materials and Methods
A case control study was carried out at Quaid-e-Azam University, Islamabad in collaboration with Jamila Sultana Foundation Rawalpindi, Thalassemia House Rawalpindi and Pakistan Institute of Medical Sciences (PIMS), Islamabad from 5th January, 2010 to 5th December 2014. The patients selected for the study were diagnosed as beta thalassemia major according to Hemoglobin electrophoresis. These patients were on regular blood transfusions with chelation therapy (desferroxamine injections). Patients suffering from any blood disorder other than beta thalassemia major or any other pathology besides spleen and liver enlargement or hepatitis B and C were not included. Total 300 individuals out of which 200 were patients suffering from beta thalassemia major and 100 were control matched for age and gender.

The age of thalassemic patients along with their corresponding control included in the study was between 8 to 22 years. Informed consent and a detail proforma including history and clinical examination were filled on patients visit to the thalassemia center for blood transfusion with chelation therapy. Height in centimeter and Weight in kilogram were measured and BMI was calculated according to the following formula:

\[ BMI = \frac{\text{Weight in kilogram}}{\text{Height in meters}^2} \]

The blood samples from controlled individuals were collected in hospital and blood from thalassemic patients were collected when they came for their routine blood transfusions with chelation therapy. For collection of blood sample, the sampling area was cleaned with a spirit swab. Blood sample of (3ml) was drawn from the right median cubital vein of both female and male patients and control individuals. Blood was then collected in labeled serum separator tubes containing Ethylene diamine tetra acetic acid (EDTA). The blood samples were centrifuged at 3000 rpm for 10 minutes, and serum separated was stored at 2 - 80°C until analyzed. Quantitative measurement of Hemoglobin (gm/dl) was done by and serum Ferritin was measured by using Ferritin (FTL) ELISA (Enzyme-Linked Immunosorbenent Assay) technique. Mean ± SEM of data was calculated and analyzed through Graph Pad Prism 5.01. Comparison amongst BMI, Hemoglobin and serum Ferritin levels with the control group was done by using unpaired t-test. Non parametric (Spearman) correlation coefficient was used to find the correlation between BMI and Ferritin and Hb levels. P<0.05 was considered statistically significant in both cases.

Results
The results of present study for the following variables are:

**Age**
Mean ± SEM of age in male and female patients of <13 years was 10.3± 0.20 years. Male patients ≥13 years had Mean ± SEM of age 16.7 ± 0.42 years, whereas the female patients of ≥13 years had Mean ± SEM of age 17.8 ± 0.70 years.

**Height (cm)**
All four groups of thalassemia patients showed significantly reduced (P<0.001) height in comparison with their corresponding control group. Comparison of height (cm) of male and female thalassemic patients with their corresponding control of different age groups is represented in Fig 1.

**BMI (Kg/m2)**
Comparison of Body Mass Index (Kg/m2), in control
and thalassemic female and male patients of different age groups is shown in Fig 2. All four groups of thalassemia patients showed significant reduction (P<0.001) in BMI on comparison with their corresponding control group.

Correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) levels

While thalassemic females of ≥13 years, BMI (Kg/m2) had a significant (P<0.001) positive correlation with Hemoglobin (gm/dl), (r=0.558). While thalassemic males of <13 years had a significant (P<0.001) negative correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) levels (r=-0.374).

On calculating correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) in <13 years thalassemic males it was concluded that there was a significant (P<0.01) negative correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) levels (r=-0.374). Correlation of BMI (Kg/m2) with Hemoglobin (gm/dl) in control and thalassemic female and male patients in different age groups is represented in Table I.

Correlation of BMI (Kg/m2) with Serum Ferritin (ng/mL) levels

While thalassemic females of ≥13 years, BMI (Kg/m2) had a significant (P<0.001) positive correlation with Serum Ferritin (ng/mL), (r=0.558). While thalassemic males of <13 years had a significant (P<0.001) negative correlation of BMI (Kg/m2) with Serum Ferritin (ng/mL) levels (r=-0.374). Correlation of BMI (Kg/m2) with Serum Ferritin (ng/mL) in control and thalassemic female and male patients in different age groups is represented in Table I.
patients in different age groups is shown in Table I. Thalassemic females of \( \geq 13 \) years had a significant (\( P<0.001 \)) positive correlation with serum Ferritin (ng/mL) levels \( * 0.498 \).

Table I: Correlation of BMI (Kg/m2) with serum Ferritin (ng/mL) and Hemoglobin (gm/dl) levels in thalassemic female and male patients of different age groups

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (Years)</th>
<th>Groups</th>
<th>Hemoglobin (gm/dl)</th>
<th>Ferritin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 13</td>
<td>Thalassemic n=50</td>
<td>-0.110</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td>( \geq 13 )</td>
<td>Thalassemic n=50</td>
<td>0.558***</td>
<td>0.498***</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td>Thalassemic n=50</td>
<td>-0.374**</td>
<td>-0.188</td>
</tr>
<tr>
<td></td>
<td>( \geq 13 )</td>
<td>Thalassemic n=50</td>
<td>-0.238</td>
<td>0.127</td>
</tr>
<tr>
<td></td>
<td>&lt; 13</td>
<td>Thalassemic n=50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \geq 13 )</td>
<td>Thalassemic n=50</td>
<td></td>
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</tr>
</tbody>
</table>

Discussion

In our study we observed that patients suffering from thalassemia major presented with reduced height and weight which was associated with increased serum ferritin and low hemoglobin levels. Najaf et al., (2008) research revealed that 70% of the males and 73% of female thalassemic patients of 10–27 years suffered from short stature.\(^{15}\) While Li et al. (2002) observed short stature in 29.7% of patients. The iron overload leading to endocrinopathies, chronic anemia, zinc and folate deficiencies can lead to short stature.\(^{16}\) These findings are in accordance to our study results in which we observed reduced height in all four groups of thalassemic males and females patients. Therefore, close observation of growth in such individuals can lead to early detection of such findings can be managed to their full extent so, that the individual achieve their normal adult height.\(^{17,18}\)

Patients with thalassemia major are exposed to many growth abnormalities as an outcome of the disease or due to the adverse effects of chelating therapy which they receive on regular basis as described.\(^{15}\) Work done by Ali and Hamdollah, (2004) on thalassemic patients revealed that reduced BMI was more apparent in greater than 10 years of age, 20 which are similar to our study results. Thalassemic males of \(< 13\) and \(\geq 13\) years and thalassemic females of \(\geq 13\) years in our study had reduced BMI as compared to the control group. The explanation to these results can be that endocrinopathies which appear as a result of iron overload and development of side effects due to prolong use of chelation therapy can be chief contributing factors in development of underweight thalassemic patients.\(^{20}\) Deena et al. (2014) also showed similar results of 18 (30%) patients who had low BMI of more than 12 years of age.\(^{21}\) This finding is indicating that low BMI is highly dependent on disease progression and are in accordance with our present findings.

Viprakasit et al. (2001) explained that frequent blood transfusions normally reestablishes the normal growth spurt.\(^{22}\) However, despite frequent blood transfusions the adolescent growth spurt is often delayed, except if rigorous iron chelation treatment is commenced at an early age in life.\(^{23}\) Previous studies on thalassemic patients revealed that average age of 12 ± 8 years occasionally suffered from growth failure as 77.4% of these patients had normal BMI.\(^{24}\) Although these results are contrary to our study findings where low BMI and reduced height was detected.

Shalitin et al. (2005) also observed that thalassemic patients receiving effective chelation therapy in prepubertal years still developed short stature with significantly raised serum Ferritin levels.\(^{25}\) But these finding were contrary to results obtained by De Sanctis et al. (1994) who detected no significant difference in final height between patients who started chelation therapy during adolescence with high serum Ferritin level and those who started chelation therapy during childhood with low serum Ferritin levels.\(^{17}\)

Hegazi et al. (2013) observed a significantly low Hb levels and red blood cell count along with significant increase in the mean serum levels of iron and Ferritin in thalassemic patients as compared with control groups.\(^{26}\) These findings are in accordance with results obtained by Charles and Linker, (2005); who also reported that Hb levels in thalassemic patients are significantly lower than control.\(^{27}\) These results are similar to our study findings as all thalassemic groups had low Hb levels as compared to the control groups.

Hegazi et al. (2013) carried out a study on thalassemic male and female patients of 4-18 years of age, where there was a significant increase in the mean serum levels of iron and Ferritin in thalassemic patients as compared to control groups.\(^{28}\) Similarly, Abdulzahra et al. (2011); work also revealed that iron indices were markedly increased in thalassemic
patients, and the mean serum level of Ferritin were also raised as compared to control group. Similarly, in our study high serum Ferritin levels were observed in all four thalassemic groups as compared to the control groups which was similar to the results reported by Adil et al. 2012, suggesting that increased serum Ferritin levels are related to short stature and endocrinopathies.

**Conclusion**

In beta thalassemic patients growth disturbance or delay is main clinical feature that affects the life and wellbeing of such individuals. Our study has revealed that patients with beta thalassemia suffer from reduced height, BMI which is enhanced in patients having high levels of serum Ferritin (ng/mL) and low Hemoglobin (gm/dl).

Under-nutrition and complications of thalassemia such as tissue hypoxia and side effects of chelating therapy with desferrioxamine effect the patients with iron overload. Therefore, lifelong care and management of such patients is mandatory which requires significant cost for proper treatment and ruling out other factors like various hormones that might play a role in development of short stature.

**REFERENCES**