Splenectomy as an Alternative Way to Ameliorate some biomarker in β-Thalassemia patients

Zainab Salman Mahdi Al-Jobouri, Abeer Cheaid Yosif, Israa Mustafa Salih

University of Kerbala, College of Applied Medical Sciences, Department of Clinical Laboratory

Abstract

β-thalassemia is a spectrum of hemoglobinopathies with clinical manifestation of mild to severe anemia and need of serious therapy treatment. The present study was to evaluate the effect of splenectomy on some parameters in male patient with blood transfusion dependent β-thalassemia. In this study 100 individuals was used and divided as follow: A seventy male patients (35 without splenectomy and 35 with splenectomy) with blood transfusion dependent β-thalassemia against 30 healthy subjects with mean ages (21.46±4.22) were enrolled. The present result found significant decrease(P≤0.05) for leptin, LH, FSH and testosterone in thalassemic patients groups as compared to control group, while there was significant increase (P≤0.05) in serum Ghrelin concentration in thalassemic patients group as compared to control group. The present study conclude that Leptin and ghrelin have a considerable role in sexual maturity and fertility observed in thalassemia male patients. Splenectomy has the ability to reflect the negative changes caused by thalassemia. This effect is not only on metabolic hormones, but it’s highly effective for fertility and normalization if reproductive problems in adult male patients.

Key words: β-Thalassemia, leptin and ghrelin.

Introduction

β-thalassemia is one of most common genetic disorders characterized by abnormal synthesis of beta globin chain of hemoglobin’s molecule which is caused by the reduced or absent synthesis of the beta globin chain (Suman et al., 2017). Supportive treatment of patients with thalassemia major (TM), including regular blood transfusions along with iron chelation and increased iron absorption from the digestive tract, causes excess iron in the body (Rismayanti et al., 2017). Iron overload which lead to fully saturated transferrin, which in turn increases circulating non transferrin-bound iron (NTBI). Hence, NTBI will enter the cells through certain channels within the cell, labile iron pool will generate Reactive Oxygen Species (ROS) through Fenton reaction and ultimately damage the cell(Knovich et al., 2009; Salibaet al., 2015).

Complications in β-thalassemia patients such as iron deposition in vital organs such as liver, cardiac, endocrine glands occurred regardless of adequate chelation therapy; Hypogonadism is the most frequently reported endocrine complication caused by iron deposit in hypothalamus, pituitary, and gonads (De Sanctis, et al., 2017). Leptin is produced and secreted predominantly from adipose tissue into the circulation; the levels circulating leptin reflect adipose tissue size and also change with nutritional state (Münzberg and Morrison, 2015). leptin is considered as a pleiotropic hormone that regulates not only bodyweight but many other functions, including vascular function, bone and cartilage growth, immune system and systemic inflammatory response as well as the normal physiology of the reproductive system (Behnes et al. 2012; Pérez-Pérez et al. 2015). In a state of decreased energy supply, a decreased leptin level has been found to be a key mediator of neuroendocrine abnormalities seen in hypogonadotropic Hypogonadism.
Ghrelin is a gut peptide composed of 28 amino acids mostly secreted in the gastric fundus mucosa (Mihalache et al., 2016). The wide distribution of ghrelin receptors implies its broad physiological effect, ghrelin is known for its role in the stimulation of appetite and feeding behavior, energy homeostasis, and carbohydrate metabolism, ghrelin’s orexigenic (appetite-stimulating) effects get mediated via metabolic need-driven homeostatic feeding as well as central actions on reward, memory, and motivated feeding behavior (Makris et al., 2017). Also, ghrelin has been suggested as a potential modulator of reproductive functions. Ghrelin finds relevance in neuroendocrine integrators for concomitant control of energy balance and reproductive functions, along with other versatile metabolic and neuro-modulatory hormones such as leptin (Sengupta et al., 2019).

The aim of the present study was to demonstrate the effects of splenectomy on leptin, Ghrelin, and some reproductive hormones levels in male patients with blood transfusion dependent β-thalassemia.

**Materials and methods**

- **Study design:** One hundred Iraqi males (30 controls and 70 patients) with age range between (15-30) years old were involved in this study. The 70 patients were divided into 2 subgroups (35 patients without splenectomy and 35 with splenectomy). All patients were with blood transfusion dependent β-thalassemia. These patients were registered as β-thalassemic patients in "Thalassemia Unit" at (Babel Maternity & Children) in Babylon city, (Iraq).

- **Blood collection:** 3 ml of blood were collected from individuals in the morning in Serum Separator Tubes (SST) and the serum separated by centrifugation (4000 RPM for 10 minutes).

- **Hormones analysis:**
  All groups assessed for some serum reproductive hormones

  - **LH:** the evaluation of LH ELIZA kit was performed as mentioned the manufacturing company (ACCUBIND Co., USA); that depended on the technique of the quantitative determination of LH concentration in human serum by a microplate enzyme immunoassay, colorimetric.

  - **FSH:** the evaluation of follicle FSH ELIZA kit was conducted according to the manufacturing company (ACCUBIND Co., USA); that depended on the quantitative determination of FSH concentration in human serum by a microplate enzyme immunoassay, colorimetric.

  - **Testosterone Hormone:** the evaluation of testosterone hormone ELIZA kit was executed as mentioned the manufacturing company (ACCUBIND Co., USA); that depended on the quantitative determination of total testosterone concentration in human serum by a microplate enzyme immunoassay, colorimetric.

- **Biomarkers Analysis:**
  - **Leptin:** evaluating of Human Leptin ELIZA Kit was performed as mentioned the manufacturing company (BT-LAB Co., China) that dependent on the technique of accurate quantitative detection of human Leptin in serum.
Ghrelin: the evaluation of Human Ghrelin ELIZA Kit was conducted according to the manufacturing company (BT-LAB Co., China) that dependent on the technique of accurate quantitative detection of human Ghrelin in serum.

**Statistical analysis:**
The data was statistically analyzed through SPSS package (SPSS, Version 24). Pearson correlation and multivariate ANOVA has been used for the comparison among subdivided groups in the measured parameters, at significant $P<0.05$.

**Results and Discussion:**

![Figure (1): Comparison of ghrelin concentration among control, non splenectomy and splenectomy.](image)

$*= P \leq 0.05$

The figure (1) showed significant increase ($P \leq 0.05$) in the concentration of ghrelin for both non splenectomy and splenectomy ($4.75 \pm 0.22$ng/ml), ($3.16 \pm 0.30$ng/ml) thalassemic male patients, respectively as compared to control group ($2.36 \pm 0.26$ng/ml). There was significant decrease($P \leq 0.05$) of ghrelin concentration in splenectomized group in comparison to non splenectomy group.

The hormone Ghrelin involved in the regulation of energy homeostasis and body weight; Ghrelin increased body weight by increasing food intake (Lee et al., 2018). Therefore, weight loss(whether resulting from reduced food intake or increased energy expenditure through physical activity) is accompanied by an increase in ghrelin level(Mihalache et al., 2016). The higher serum ghrelin might be considered as a compensatory response to growth retardation or partial resistance to ghrelin that leads to its increased level(Karamifar et al., 2010).The result of present study disagreement with Majeed, (2017), who found lower values of ghrelin in
thalassemia β-major. The result also disagreement with Moshtaghi-Kashanian and Razavi, (2009) who found lower values of serum ghrelin in thalassemic patients. The present study showed significant decrease of serum ghrelin in splenectomized thalassemic patients in comparison to non splenectomy group, this result can be explained by that variations in body weight, were observed to lead to compensatory responses of ghrelin levels(Delporte, 2013). As thalassemic patients gained weight after splenectomy; Therefore, weight gain is accompanied by a decrease in ghrelin levels; reduced ghrelin secretion is found to be an adaptive mechanism to a long-term positive energy balance(Mihalache et al., 2016).

![Figure (2): Comparison of leptin concentration among control, non splenectomy and splenectomy.](image)

* = P≤0.05

The figure (2) showed significant decrease (P≤0.05) in leptin concentration for both non splenectomy (1.90±0.11ng/ml) and splenectomy (2.99±0.17ng/ml) thalassemic male patients as compared to control group (3.57±0.25ng/ml). While, there was significant increase (P≤0.05) of leptin concentration in splenectomized group in comparison to non splenectomy group.

The expression for this decrease in leptin serum level among thalassemia patients can be related to the toxic effects of iron overload on cell membranes and proteins; since free iron causes peroxidative damage in lipid membrane and proteins with the generation of free radicals, which results in the destructions of the adiposities and the dysfunction in adipose tissue (Elsayh et al., 2016). This results in agreement with Al-Naama et al., (2016), HAGAG et al., (2018) and Harbi et al., (2020) who found significantly lower serum leptin level in thalassemia patients as compared with control group.

In the present study there was a significant increased of leptin
level among splenectomy group as compared to non-splenectomy group. This result in agreement with Rosa et al., (2015) who found that the splenectomized animals showed greater serum leptin and insulin. In the fact the leptin expression is stimulated by cytokines, such as TNF-α and IL-1; splenectomy results in the expression of IL-1 and TNF-α in the liver, which leads to fever and possibly stimulates leptin production (Feleder et al., 2003).

Figure (3): Comparison of Luteinizing Hormone (LH) concentration among control, non-splenectomy and splenectomy.

* = P≤0.05
Figure (4): Comparison of Follicle Stimulating Hormone (FSH) concentration among control, non splenectomy and splenectomy

*= P≤0.05

The figure (3) showed significant decrease (P≤0.05) in the concentration of luteinizing hormone for both non splenectomy and splenectomy (1.95±0.35 mlU/ml), (3.18±0.29 mlU/ml) thalassemic male patients, respectively as compared to control group (4.73±0.26 mlU/ml). There was significant increase (P≤0.05) of luteinizing hormone concentration in splenectomized group in comparison to non splenectomy group.

The figure (4) demonstrated significant decrease (P≤0.05) in the follicle stimulating hormone concentration for both non splenectomy (2.91 ±0.91mlU/ml) and splenectomy (5.22 ±0.41mlU/ml) thalassemic male patients as compared to control group (10.46 ±0.61mlU/ml). While, there was significant increase of follicle stimulating hormone concentration in splenectomized group in comparison to non splenectomy group.

The decrease of LH and FSH might be due to iron overload which lead to fully saturated transferrin, which in turn increases circulating non transferrin-bound iron (NTBI). Hence, NTBI

Figure (5): Comparison of testosterone concentration among control, non splenectomy and splenectomy groups.

*= P≤0.05

The figure (5) illustrated significant decrease (P≤0.05) in the concentration of testosterone for both non splenectomy (0.74±0.20 ng/dl) and splenectomy (1.29±0.10ng/dl) thalassemic male patients as compared to control group (2.03±0.26 ng/dl). On the other hand, there was significant increase of testosterone concentration in splenectomized group in comparison to non splenectomy group.

www.turkjphysiotherrehabil.org
will enter the cells through certain channels within the cell, labile iron pool will generate Reactive Oxygen Species (ROS) through Fenton reaction and ultimately damage the cell (Knovich et al., 2009; Saliba et al., 2015). The decreased levels of testosterone might be due to reduced GnRH secretion lead to inadequate pituitary stimulation, this could be the cause for the detection of decreased levels of gonadotropins resulting in low circulating levels of gonadal testosterone (Majeed, 2017). Also decreased levels of testosterone may be due to the effect of iron, in particular that of NTBI on the testes; histological examination of testicular tissues from autopsies demonstrated testicular interstitial fibrosis with small, heavily pigmented, undifferentiated seminiferous tubules and an absence of Leydig cells (Srisukh et al., 2016). In the classic knowledge, iron accumulated in the pituitary gland of thalassemia patients results a cytotoxic effect. These cytotoxic effects result to hyporesponsiveness of pituitary gland to GnRH and lead to hypergonadotrophic Hypogonadism (WANG, Tso and TODD, 1989), which can appear as low estradiol or testosterone with LH and low FSH (Srisukh et al., 2016). The present results in agreement with Srisukh et al., (2016) who found that high iron deposition in the pituitary gland especially the anterior part result in damaged gonadotrophs and decreased gonadotrophin (LH and FSH) production. Hegazi et al., (2013) and Srisukh et al., (2016) who found significantly lower serum LH and FSH levels in patients compared with control group. Also, the result of present study in agreement with Khan, (2017), Adiwinoto et al., (2020), who found lower serum testosterone in thalassemia patients as compared to control group. The results of present study showed significant increase in the concentrations of luteinizing hormone, follicle stimulating hormone and testosterone among splenectomized thalassemic patient as compared to non splenectomy thalassemia patients, might be explained by the stimulatory effect of leptin on gonadotropin releasing hormone (GnRH) secretion and the activity of the hypothalamic-pituitary-gonad (HPG) axis (Garcia-Galiano, Allen and Elias, 2014). Therefore, increased Gonadotropin hormones (LH and FSH) and testosterone could be related to increased serum leptin among splenectomized thalassemic patients of present study.

**Conclusion:**
Leptin and ghrelin have a considerable role in sexual maturity and fertility observed in thalassemia male patients. Splenectomy has the ability to reflect the negative changes caused by thalassemia. This effect is not only on metabolic hormones, but it’s highly effective for fertility and normalization if reproductive problems in adult male patients.

**References:**
Physiology, 284(6), pp. R1466–R1476.


