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Serum Level of Preptin Hormone in Children with Type-1 Diabetes Mellitus

ABSTRACT:

Background Preptin is a hormone which is calm of 34-amino acids (MS 3948 kDa). It is a peptide that is secreted along with insulin and amylin from the β-cells of the pancreas.

The aim of the present study was to estimate the serum level of preptin hormone in children with type I diabetes mellitus getting an insulin therapy.

Patients & Methods: In this study, a sample which consisted of 35 children with type I diabetes mellitus receiving a treatment of insulin therapy (ages range from 7 to 12 years) and 30 apparently healthy children of a comparable age. Preptin hormone was measured via ELISA, while the blood glucose was measured by UV-VIS spectrophotometer.

The Results: The result of this study reveals that preptin has a mean serum level of 82.79 ± 4.3 ng/ml in children with type-I diabetes mellitus, while its serum level in control group was 138.62 ± 6.1 ng/ml. It is also found in this study that the BMI of the studied group lower than the control children. In conclusion, preptin is found to be significantly lower in children with type-I diabetes mellitus in comparison with the control group.

Conclusions: In conclusion, the preptin hormone was found lower in children with type-I diabetes mellitus in comparison with non-diabetic children.

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Introduction

Diabetes is combinations of heterogeneous disorder that usually presenting as an episode of hyperglycemia and disorder of glucose metabolism, as a result of the absence of insulin, which is synthesized and recreated by by the pancreas. Insulin maintains glucose entry to human body cells. In diabetes mellitus type-1, the pancreas does not synthesized enough insulin. [1]

Preptin is an anabolic hormone mainly maintains bone and may lead to the preservation of bone quantity that experimental in hyperinsulinemia. [2] Preptin is a bone active peptide hormone that may act in concert with the other β-cell hormones insulin and amylin to stimulate bone foundation in hyperinsulinemia such as in obesity. [3]. Pancreatic β-cell destruction in patients with type I diabetes

mellitus avoids the secretion of insulin and preptin, whereby falling their effects on the RUNX2 gene. This reduction decreases the proliferation and the differentiation of primitive cells into osteoblasts and hence to their resistance to apoptosis. [4]

The aim of the present study was to estimate the serum level of preptin hormone in children with type I diabetes mellitus getting an insulin therapy. While the objectives include:

1. To measure the serum level of preptin hormone in children with type I D.M and the control.

2. To measure the serum level of glucose in a postprandial state for the diabetic children and the control group.

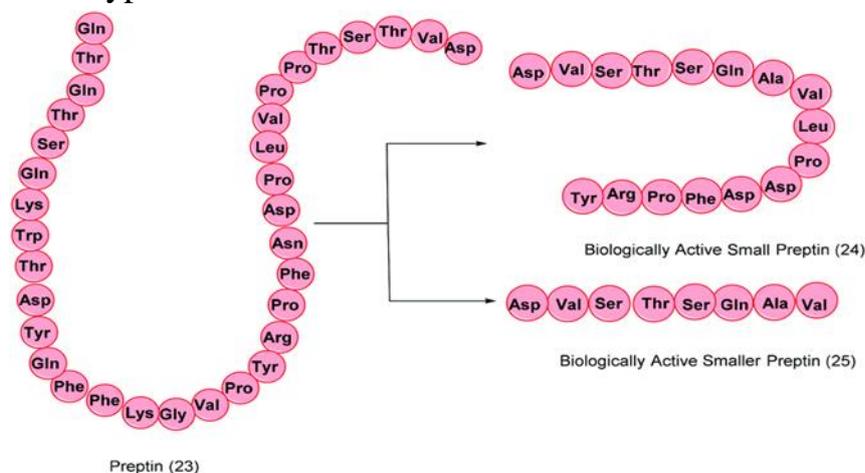3. To measure the body mass index for both the diabetic children and the control group.

Fig.(1): Sequence of human preptin.(5)

Patients And Methods

This is a cross-section study, which was conducted on 65 children with an age that was range from 7 to 12 years. Thirty-five out of 65 were type-1 diabetic patients on insulin therapy (20 males and 15 females) and the remaining 30 children were considered as a control group (15 males and 15 females) and those were looks apparently healthy. Children with diabetes mellitus were collected from a primary health center and a private clinic under a supervision of a pediatrician from the period of 1st of October 2015 till the end of July 2016 in Tikrit City. The patients and healthy individuals were subjected to a questionnaire and physical measurement containing the following:

- Age in years.
- Weight in kilograms.
- Height in centimeters.

Disposable syringes and needles were used for blood collection. A random blood samples were aspirated from both the patients and the controls, which constitute of 2 mls of venous blood. After allowing the blood to clot at room temperature for 15 min, blood samples were centrifuged at 3000 rpm for 10 min. 0.7 ml of sera were separated, divided in aliquots and frozen at -10 ^{0}C for estimation of the serum level of glucose to insure about the diagnosis of type-1 diabetes mellitus [6] and preptin hormone. Samples showing hemolysis were discarded. [7, 8]

I. Determination of serum glucose:

Serum glucose level was measured by glucose kit (BioMerieux, France), using an enzymatic method (Glucose-oxidase method). [9]

II. Determination of preptin hormone:

Principle: The kit contains an enzyme linked immune sorbent assay (ELISA) created on the sandwich technology to assay the human preptin, with adding preptin to the pits, which were pre-coated with preptin monoclonal antibody and then nurtured. Anti preptin antibodies were added and categorized with biotin to unite with streptavidin-HRP, this grouping forms immune complex. The enzyme that is unbound was removed by washing. Substrate (a) and (b) were added. The solution was blue in color and change into yellow with the effect of acid. The strength of the color and the concentration of human preptin are positively correlated.

Procedure:

The standard solutions were diluted as follow:

150 Nano gram /Litter	standard no.1	120 microliter standard no. 2 + 120 microliter **standard dil.**
300 Nano gram /Litter	standard no.2	**120 microliter standard no.3 + 120 microliter standard dil.**
600 Nano gram /Litter	standard no.3	**120 microliter standard no.4 + 120 microliter standard dil.**
1200 Nano gram /Litter	standard no.4	**120 microliter standard no.5 + 120 microliter standard dil.**
2400 Nano gram /Litter	**standard no.5**	**120 microliter original standard + 120 microliter standard dil.**

1. The number of stripes that were needed was determined by number of samples
    to be tested. Each standard solution and every blank well should be arranged
    with three or more wells as much as possible.

2. Injection of the sample:
    A. Blank well: no sample was added, and an anti preptin antibody labeled
        with biotin or streptavidin-HRP is added
    B. Standard solution well: 50 µls of standard and 50 µls of streptomycin-
        HRP were added.
    C. Sample well: 40 µls of the sample was added and then 10 µls of
        preptin antibodies, finally 50 µls of streptavidin-HRP were also added .
            Then the tubes were covered with seal plate membrane. The tubes
        were gently shaked and mixed and incubate at 37°C for 60 minutes.

3. Washing solution: The solution were diluted (30 X) with distilled water
    for later use.

4. The washing: The seal plate were uninvolved carefully, the fluid was drain and
    shake off the remaining liquid. Each well was filled with washing solution. The
    liquid was drain after 30 seconds standing. This method was repeated five times.

5. Development of the color: 50 µls of chromogen solution A were added firstly to
    each well and then 50 µls of chromogen solution B were added to each well as
    well. The wells were gently

shaked and mixed, and then incubate for 10

minutes at 37°C away from the light for the color development.

6. Termination of the reaction: 50 µls of rest solution were added to each well to rest

the reaction, the blue color changes to yellow instantly at that time.

7. Assay procedure: The blank well were adjusted at a zero, then the absorbance

of each well was measured one by one at 450 nm wavelength, this step

should be carried out within the 10 minutes after g added the stop solution.

8. Calculation: The applications and the corresponding OD values were planned

and the linear regression equation was used to draw the standard curve. Then

according to the absorbance values of the samples, the concentration of the

corresponding samples were calculated..

Analysis of the results:

The results were obtained as mean ± SD. t-test was used for the assessment of the results concerning patients and the control groups. Significant variations between the studied groups were considered when P value was 0.05 and less.

Results:

It is evident from this study that the children affected with type-1 diabetes mellitus had a significantly lower serum level of preptin hormone (82.79 ± 4.3 ng/ml) compared with the control group (138.62 ± 6.1 ng/ml) at a p value of fewer than 0.05. While there was no significant difference concerning the body mass index between the patients (24.31 ± 0.54 kg/cm^2) and the control (26.18 ± 0.57 kg/cm^2). The serum glucose level was used to insure that the studied group was affected by diabetes mellitus type-1.

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Table (1): The mean levels of body mass index, serum glucose, and serum preptin level along with the standard deviation for each of them.

Parameters	Patients (no. = 35)	Control (no. = 30)	P value
BMI	24.31 ± 0.54 kg/cm^2	26.18 ± 0.57 kg/cm^2	Not Sig.
Serum glucose	284.88 ± 12.6 mg/dl	81.62 ± 9.11 mg/dl	0.008**
Serum preptin	82.79 ± 4.3 ng/ml	138.62 ± 6.1 ng/ml	0.022*

Table (2): The correlation of the studied markers in children with type-I diabetes mellitus.

Parameters	BMI	Serum glucose	Serum preptin
BMI	-	- 0.026*	-
Serum glucose	-	-	- 0.008**
Serum preptin	+ 0.041*	-	-

Discussion

The estimation of serum preptin was significantly (P < 0.05) higher in control in contrast with the patients collection (table 1). Pancreatic islet beta-cells secrete the preptin along with insulin and amylin. Preptin may enhance the insulin-like growth factor receptor linked to the protein kinase to enhance the calcium-dependent insulin secretion under great glucose levels. This result may provide a new insight about the autocrine action of preptin. (10) It had been suggested that unstable blood glucose concentrations similar those seen during the post-prandial hyperglycemic condition in people with type I-DM, which may contribute to a significant oxidative stress – even more than that seen in chronically elevated blood glucose.(11)

Dysfunction of the mitochondria could be one of

many chief underlying imperfections that linking obesity to type I diabetes, by decreasing insulin sensitivity and by compromising β-cell function of pancreas. $^{(12-14)}$ the results clears that there is a negative correlation between preptin hormone level and glucose level in patients group, (table 2). Preptin level may decreased with insulin level decreasing therefor it decreases with diabetic patients and increases with controls group with increasing insulin concentration.

The estimation of serum glucose was significantly (P < 0.01) grander in patients with diabetes compared to control. Hyperglycemia may lead to tissue damage through different mechanisms that includes the increased flux of glucose and other monosaccharides through the polyalcohol pathway, that increases the intracellular formation of advanced glycosylation products (AGPs), the increase in the expression of the receptor for AGPs leads to an activation of protein kinase C isoforms, and an increase in the activity of the hexosamine pathway.[15]

Conclusion, diabetic children reveals a low serum level of preptin hormone. Therefor it is recommended to study the preptin hormone in a fasting state along with the serum level of insulin to find out its correlation with insulin level.

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