

# Asprosin Role for Obese Male Patients with Diabetic Mellitus Type II

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## Abstract

Hormones, their receptors, and the associated signaling pathways make compelling drug targets because of their wide-ranging biological significance to study the role of asprosin in obese male patients with diabetic mellitus type II. ELISA method was used to assay asprosin and insulin. Blood was taken with drawn sample from 30 obese normal patients with age range (40-60) years, 30 diabetic patients with age range (40-60) years at duration of disease (1-5) years and 30 normal healthy patients. The mean difference between T2DM according to insulin % ( $23.8 \pm 0.6$ ) was increased than the mean of IFG ( $17.7 \pm 1.0$ ) (P 0.000). The mean difference between T2DM according to asprosin ( $122.1 \pm 21.8$ ) was increased than the mean of IFG ( $51.4 \pm 2.7$ ) (P 0.000), the mean differences between DM2 and IFG cases in different weight groups (Ob., Ow. and Nw) according to insulin was studied, the results showed that, there were significant differences in DM and IFG obese groups (G1 and G2) according to insulin ( $24.18 \pm 1.13$ ,  $15.56 \pm 0.66$ ) P (0.00), however, there were significant differences between DM and IFG in Normal weight groups (G5 and G6) according to insulin ( $19.98 \pm 0.93$ ,  $11.12$ ) P (0.00), while no significant differences between DM and IFG in Over weight groups (G3 and G4) according to insulin ( $27.22 \pm 0.34$ ,  $28.56 \pm 1.59$ ) P (0.42). The mean differences between diabetic mellitus type 2 and impaired fasting glucose cases in different weight groups (obese, over weight and normal weight) according to Asprosin were shown in Table (3), Figure (). The results showed that, there were significant differences between DM and IFG in obese groups (G1 and G2) according to Asprosin ( $307.42 \pm 8.4$ ,  $66.3 \pm 2.2$ ) P (0.00), However, there were significant differences between DM and IFG in overweight groups (G3 and G4) according to Asprosin ( $28.3 \pm 0.5$ ,  $51.7 \pm 3.2$ ) P (0.00) In addition to that, there were significant differences between DM and IFG in normal weight groups (G5 and G6) according to Asprosin ( $30.5 \pm 1.7$ ,  $21.2 \pm 1.6$ )

**Keywords:** *Asprosin, obese patients, Diabetic Mellitus Type 2, IFG.*

## Introduction

T2DM accounts for between 90% and 95% of diabetes, with highest proportions in low- and middle income countries<sup>(1)</sup>. It is a common and serious global health problem that has evolved in association with rapid cultural, economic and social changes, ageing populations, increasing and unplanned urbanization, dietary changes such as increased consumption of highly processed foods and sugar sweetened beverages, obesity, reduced physical activity, unhealthy lifestyle and behavioural patterns, fetal malnutrition, and increasing fetal exposure to hyperglycemia during pregnancy. T2DM is most common in adults, but an increasing number of children and adolescents are also affected<sup>(2)</sup>. The major causative of T2DM is due to the combination

of disorder in insulin secretion via pancreatic  $\beta$  cells and peripheral insulin resistance (IR)<sup>(3)</sup>. IR as a result of defects within signaling pathways of insulin in its target tissues<sup>(4)</sup>. Insulin is a peptide hormone made up of 51 amino acids (a.a), it is created by the  $\beta$ -cells of the pancreas. Insulin peptide is primarily produced as preproinsulin and fractionate into proinsulin and later split into two peptides are C-peptide and insulin<sup>(5)</sup>. Numerous factors are known to affect insulin formation. The unique and most significant physiological method that stimulates the process of transcription and translation at gene of insulin and RNA respectively is metabolism of glucose<sup>(6)</sup>. In response to elevated glucose in the blood after ingestion and absorption from food, insulin is secreted in two phases and connects to insulin

receptors expressed in muscle, liver and adipose tissue. The secretion of insulin from  $\beta$ -cells of the pancreas is induced by elevation level of glucose mainly of fatty acid and amino acids in the blood. In both humans and rodents, after ingestion and absorption of food, glucose triggers a higher insulin response relative to other nutrients<sup>(8)</sup>, the same amount of fat or combination of fat and protein results in just about a twofold elevation in plasma insulin level relative to the basal level<sup>(8)</sup>.

## Materials and Method

This study performed during period from September 2019 to December 2019 the subject were selected from Teaching Hospital/Medical City. Questionnaires were filled by participants and to get the agreement to participants in this study to collect the information of control and patients group. Blood samples were collected from control and patients group. The sample was drawn from the vein and stored by using (5mL) disposable syringe, all samples were collected in fasting status. The sample was keep into dispensable tubes containing a gel which facilitate the separation processes of serum and allowed to clot at 37°C approximately at ten-fifteen min and then centrifuged at 2000 Xg for ten-fifteen min then the serum was stored at (-20°C) until analysis (serum insulin and asprosin).

**Subjects (patients and control groups):** Subject were enrolled in this study to three group First group: patients 30 normal obese male with age range (40-60) years. Second group: DM type 2 (30) male with age range (40-60) years the duration of disease (1-5) years. Third group: 30 normal healthy male documented by physician or lab investigation matched in their age in both obese group.

**Exclusion Criteria:** Any Patient with the following problems was excluded from the current study. with renal dysfunction. Patient with heart diseases and hypertension. Patient with thyroid disorder. Insulin drug dependency. Any person with the chronic liver disease. Type 1 diabetic patient.

**Inclusion Criteria:** According to American diabetes association ADA criteria all patients are classified as DM type 2 by measurement blood glucose and HbA1c (ADA diabetes care 2019 Jan, 42:s13-s28). Also regard to WHO criteria all patients are classified according BMI.

**Measurement of Human asprosin<sup>(9)</sup>:** Standard wells, a volume of 50 $\mu$ l of the standard solutions were

added to the standard wells. Then a volume of 10 $\mu$ l of the sample was added followed by 40 $\mu$ l of sample diluent was added to the testing sample well, in blank nothing to add. After that, a volume of 100 $\mu$ l of HRP-conjugate reagent was added to each well and then covered by used adhesive strip followed by incubation for sixty min at 37°C. The cover on a plate was removed and starting to wash process, the wash process was repeated for four times using 400 $\mu$ l of Wash Solution each time by an auto washer. A volume of 50 $\mu$ l of chromogen solution (A) and (B) was added to each well and mixed gently and followed by incubation period at 37°C for 15 min. This addition should be protected from light. Next a volume of 50 $\mu$ l Stop Solution was added to each well. The color in the wells converts from blue color to yellow color. If the color in the wells become green or the color change does not appear uniform, the plate should gently covered to ensure good mixing. Afterwards a microtiter plate reader was used to read the absorption within 15 min at 450 nm.

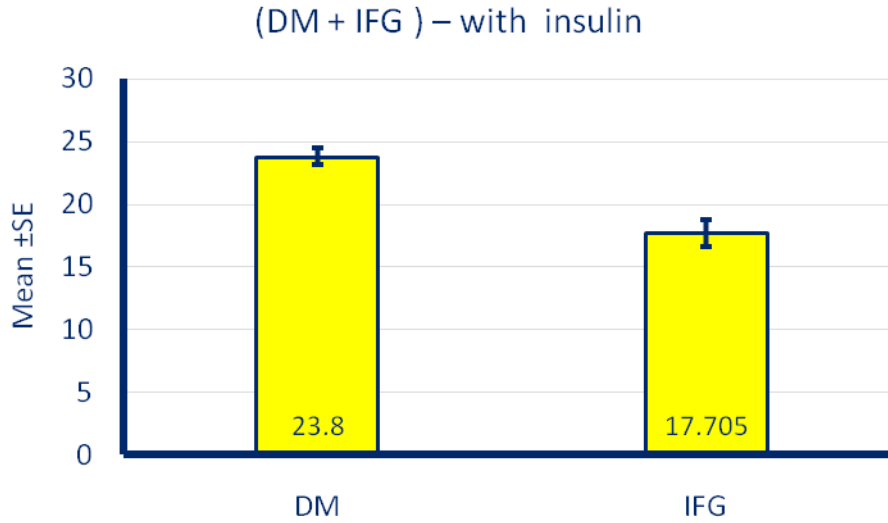
**Measurement of insulin<sup>(10)</sup>:** Standard wells, a volume of 50 $\mu$ l of the standard solutions were added to the standard wells. Then a volume of 10 $\mu$ l of the sample was added followed by 40 $\mu$ l of sample diluent was added to the testing sample well, in blank nothing to add. After a volume of 100 $\mu$ l of HRP-conjugate reagent was added to each well and then covered by used adhesive strip followed by incubation for sixty min at 37°C. The cover on a plate was removed and starting to wash process, the wash process was repeated for four times using 400 $\mu$ l of Wash Solution each time by an auto washer. After that, a volume of 50 $\mu$ l of chromogen solution (A) and (B) was added to each well and mixed gently and followed by incubation period at 37°C for 15 min. This addition should be protected from light. Then a volume of 50 $\mu$ l Stop Solution was added to each well. The color in the wells converts from blue color to yellow color. If the color in the wells become green or the color change does not appear uniform, the plate should gently covered to ensure good mixing. A microtiter plate reader was used to read the absorption within 15 min at 450 nm.

**Statistical Analysis:** The version twenty of SPSS was used to complete Statistical analysis. (Means  $\pm$  SD) were used to represent the variables. The comparison between patients group and control group was done by use student t-test; with a *p*-value of  $\leq 0.001$  was considered a significant. The method that used to find the relationship between two continuous variables was correlation coefficient (*r*).

**Ethical Approval:** Agreement from patients for sampling collection and carrying out this work is obtained from each patient.

**Results**

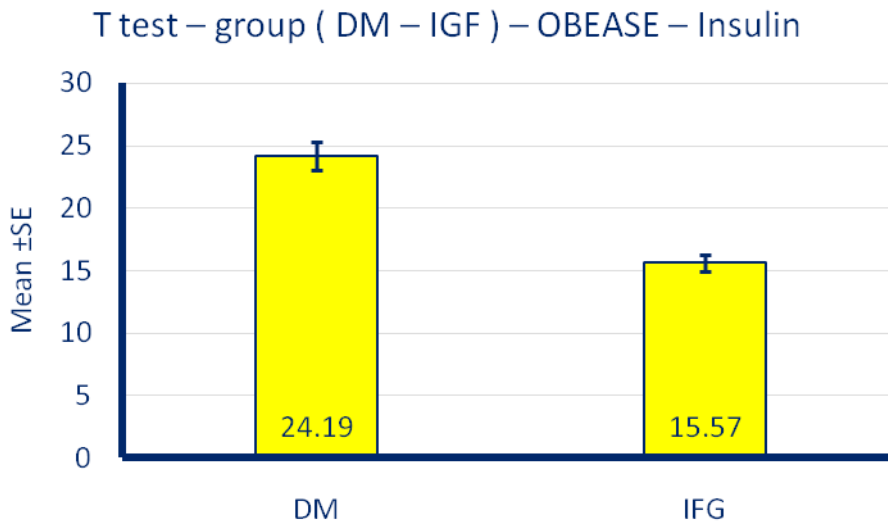
In Figure (1), the mean difference between T2DM according to insulin % ( $23.8 \pm 0.6$ ) was increased than the mean of IFG ( $17.7 \pm 1.0$ ) (P 0.000). The mean difference between T2DM according to asprosin ( $122.1 \pm 21.8$ ) was increased than the mean of IFG ( $51.4 \pm 2.7$ ) (P 0.000).



**Figure (1) Mean difference between diabetic mellitus type 2 (DM2) and impaired fasting glucose (IFG) patients according to insulin P (0.000).**

In Figures (2), the mean differences between DM2 and IFG cases in different weight groups (Ob., Ow. and Nw) according to insulin was studied, the results showed that, there were significant differences in DM and IFG obese groups (G1 and G2) according to insulin ( $24.18 \pm 1.13, 15.56 \pm 0.66$ ) P (0.00), however,

there were significant differences between DM and IFG in Normal weight groups (G5 and G6) according to insulin ( $19.98 \pm 0.93, 11.12$ ) P (0.00), while no significant differences between DM and IFG in Over weight groups (G3 and G4) according to insulin ( $27.22 \pm 0.34, 28.56 \pm 1.59$ ) P (0.42).



**Figure (2): Mean differences between DM 2 and IFG cases in Ob. Cases in different weight groups according to insulin P (0.00)**

The mean differences between diabetic mellitus type 2 and impaired fasting glucose cases in different weight groups (obese, over weight and normal weight) according to Asprosin were shown in Table (3). The results showed that, there were significant differences between DM and IFG in obese groups (G1 and G2) according to Asprosin ( $307.42\pm 8.4$ ,  $66.3\pm 2.2$ ) P

(0.00), However, there were significant differences between DM and IFG in overweight groups (G3 and G4) according to Asprosin ( $28.3\pm 0.5$ ,  $51.7\pm 3.2$ ) P (0.00). In addition to that, there were significant differences between DM and IFG in normal weight groups (G5 and G6) according to Asprosin ( $30.5\pm 1.7$ ,  $21.2\pm 1.6$ ).

**Table (3): The mean difference between DM2 & IFG cases in different weight groups (Obese, Overweight, Normal weight according to Asprosin**

Groups	Sub groups	Asprosin ng/L	P-value
Obese	DM/G1	$307.42\pm 8.4$	0.00
	IFG/G2	$66.3\pm 2.2$	
Over weight	DM/G3	$28.3\pm 0.5$	0.00
	IFG/G4	$51.7\pm 3.2$	
Normal weight	DM/G5	$30.5\pm 1.7$	0.001
	IFG/G6	$21.2\pm 1.6$	

## Discussions

These results were agreement with results obtained by<sup>(11)</sup> who found that, there were significant differences between IFG and Insulin and ( $P < 0.005$ ). Another study of<sup>(12)</sup> found that, there were significant differences between T2DM and insulin  $P < 0.001$ . Asprosin functions to increase plasma glucose levels, and circulating asprosin levels are increased by fasting (a baseline glucose condition) and decreased by feeding a high glucose condition<sup>(13)</sup>. Asprosin might be a risk factor associated with the development of T2DM. Adipose tissue has the endocrine role to regulate metabolism and balance energy homeostasis<sup>(14)</sup>. Several adipose tissue-secreted molecules can either enhance or impair insulin action<sup>(15)</sup>. Insulin resistance, a major cause of T2DM, is one of the most remarkable changes which occur with excess adiposity. Thus, obesity is causally inked to a constellation of metabolic diseases such as T2DM and metabolic syndrome<sup>(16)</sup>. Humans with insulin show pathologically elevated plasma asprosin, and its loss of function through immunological or genetic means has a profound glucose and insulin lowering effect secondary to reduced hepatic glucose release<sup>(17)</sup>. Therefore, therapeutically targeting asprosin might be beneficial in type 2 diabetes mellitus patients<sup>(18)</sup>. Hepatic glucose release into the circulation is vital for brain function and survival during periods of fasting and is modulated by an array of hormones that precisely

regulate plasma glucose levels. A fasting-induced protein hormone that modulates hepatic glucose release. It is the C-terminal cleavage product of profibrillin, and we name it Asprosin<sup>(19)</sup>. Asprosin is secreted by white adipose, circulates at nanomolar levels, and is recruited to the liver, where it activates the G protein-cAMP-PKA pathway, resulting in rapid glucose release into the circulation<sup>(20)</sup>.

## Conclusion

There were significant differences between DM2 patients according to asprosin and insulin, and there were significant differences between IFG cases according to asprosin and insulin.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

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