SERUM VISFATIN, INSULIN RESISTANCE AND BETA-CELL FUNCTION IN TYPE II DIABETIC PATIENTS AND NON-DIABETIC ADULT OFFSPRING WITH POSITIVE PARENTAL HISTORY OF TYPE II DIABETES MELLITUS

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ABSTRACT

Objectives: To determine and compare serum visfatin levels, insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B) of type II diabetic patients and non-diabetic adult offspring of type II diabetic parents with that of non-diabetic adult offspring of non-diabetic parents.

Methods: A cross-sectional comparative study conducted at Diabetes clinic of Lahore General Hospital and Physiology department of Post Graduate Medical Institute Lahore in 2018; comprised of thirty type II diabetic subjects (group III), forty non-diabetic adult offspring of type II diabetic parents (group II) and forty non-diabetic adult offspring of non-diabetic parents (group I/controls); all having an age range of thirty to fifty years. Blood pressure, BMI and waist circumference were measured. Fasting blood samples of the subjects were analyzed for serum insulin, glucose and visfatin. Insulin resistance (HOMA-IR), insulin sensitivity (HOMA-%S) and beta-cell function (HOMA-%β) were also calculated.

Results: Group III had significantly higher serum visfatin, HOMA-IR and lower HOMA-%S as compared to the controls. No significant difference was found between HOMA-%B of group III and controls. On the contrary, group II had significantly lower serum visfatin and HOMA-%S while HOMA-%β, HOMA-IR were significantly higher in comparison to the controls.

Conclusion: Visfatin production seems suppressed in non-diabetic individuals with type II diabetic parents probably due to hyperinsulinemia. Moreover, it has a little role in insulin secretion in these individuals as reflected by their higher HOMA-%B index. However, visfatin's upregulation in chronic hyperglycemia is indicative of its restorative role in the declined beta-cell function in type II diabetics.

Key words: Visfatin, nicotinamide adenine dinucleotide biosynthesis, offspring of type II diabetics, insulin resistance, insulin sensitivity, beta-cell function

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INTRODUCTION

Diabetes Mellitus has emerged as the millennium global health hazard. A deplorable statistic picture shows an upsurge of diabetes especially in the developing countries. Currently, 463 million adults 20-79 years of age are diabetic worldwide and this figure is likely to rise to 700 million in 2045. In 2019, 19.4 million adult Pakistanis were estimated to be diabetic (1). Type II encompasses majority of the diabetic cases. Its hereditary setting is complicated being polygenic in nature and environmental factors like food habits, lack of physical activity and weight gain also have a pronounced effect (2). People who have diabetes in their first-degree relatives are at double risk of developing diabetes than otherwise. When one parent

is type II diabetic, the life time risk for developing the disease will be nearly 40%. The chances of getting diabetic are further increased in case of diabetic mother. More over the risk reaches up to approximately 70% if both parents are found diabetic (3,4). Visfatin is a highly conserved pleiotropic protein, essential for survival, found in all living beings ranging from bacteria to homo sapiens. Fatty tissue, liver cells and circulating leucocytes as the main sources of its circulating levels (5). Fukuhara and his colleagues were the pioneer to learn about its adipokine function in 2005. They said that visfatin was mainly secreted by abdominal adipose tissue in humans and mice and its plasma levels were reflective of the amount of visceral fat. This adipokine binds with the insulin receptor at a different site than insulin hormone and has similar metabolic and mitogenic functions (6). It works as a cytokine, involved in the proliferation of B lymphocytes precursors in the presence of interleukin 7 and stem cell factor and was named as PBEF (7). As a key regulatory enzyme, nicotinamide phosphoribosyl transferase (Nampt), it is involved in the synthesis of nicotinamide adenine dinucleotide and performs a principal role in glucose stimulated insulin secretion (8). Although visfatin has been meticulously observed for its role in glucose metabolism, but no defined inference on its linkage with insulin resistance and beta-cell function could be ascertained so far (5). Therefore, this study was designed to determine and compare serum visfatin levels, insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B) of type II diabetic patients and non-diabetic adult offspring of type II diabetic parents with the controls.

METHODS

This cross-sectional study was conducted at the Diabetes clinic of Lahore General Hospital (LGH) and department of Physiology, Post Graduate Medical Institute, Lahore in 2018. This study included 30 type II diabetic patients (group III) and 40 non-diabetic adult offspring of type II diabetic parents (group II). The control group consisted of 40 non-diabetic adult offspring of non-diabetic parents (group I). Exclusion criteria was any acute illness for the past 2 weeks, history of chronic inflammatory disease, type I diabetes mellitus or any systemic disease, grade I hypertension, on any kind of medication, smoking and morbid obesity, BMI $\geq$ 30, (9). Females with pregnancy, history of menstrual irregularities, acne and hirsutism were also not part of the study.

The study was approved by ethical committee of Postgraduate Medical Institute Lahore. Written and informed consent was obtained from each subject. Detailed clinical history followed by general physical examination was done for each subject. Blood pressure was measured twice at the left arm in sitting position after 15 minutes rest. Weight was measured in minimal clothing and without shoes using a weight machine and height by a height chart. Following formula was applied: weight in kilograms divided by their height in $(meter)^2$. For waist circumference, reading was taken at a point midway between the lowest palpable rib and the uppermost lateral border of iliac crests using a tape measure keeping the subject in upright position with feet together and shirt removed (10).

5ml blood sample was collected from the subjects under aseptic measures after an overnight fast of 12 hours. The blood was transferred in gel activated vacutainer tubes. Tubes were then centrifuged at 3000 revolutions per minute for sera extraction. Serum glucose was determined immediately thereafter and rest of the sera was shifted in properly labelled Eppendorf tubes and frozen at -20 ^{0}C for further analysis. Serum visfatin levels were determined by direct ELISA method (Human visfatin ELISA kit, catalog # 11560, Glory science company, USA). Assay range of the kit was 1-20 µg/l, Intra-assay precision < 9%, inter-assay precision < 15% and sensitivity of the assay $\geq$ 1 µg/l. Serum Insulin levels were determined by direct Human ELISA kit, Diametra Itlay, Ref # DK0076 using an analyzer STAT FAX 303 reader. Serum glucose was analyzed using GOD-PAP enzymatic colorimetric method of Human Diagnostics, Germany kit ref # 10260. The indices for Insulin resistance, sensitivity and beta-cell function were calculated by Homeostasis Model Assessment.

HOMA-IR = fasting serum glucose (mg/dl) x fasting serum insulin (µIU/ml)/405

HOMA-%S = 1/HOMA-IR x 100

HOMA-%β = 360 x fasting serum insulin (µIU/ml)/fasting serum glucose (mg/dl) – 63 (11).

Data analysis was done by IBM-SPSS version 26. Normal distribution of the data was checked by Shapiro-Wilk's statistics and p-value <0.05 was considered to be non-normally distributed. Median with interquartile range (IQR) was given for non-normally distributed quantitative variables. Kruskal-Wallis test was applied to compare the difference in the median (IQR) between the three groups while Mann Whitney U Test for observing median (IQR) difference of the two groups. A p-value of <0.05 was considered as statistically significant.

RESULTS

There was no significant difference in age, frequency distribution of gender and BMI of the three groups. However, waist circumference of group II females was significantly higher than the females of the control group (group I) (p-value <0.05). No significant difference in waist circumference of males or females of other groups was obtained (Table-1).

Fasting levels of serum visfatin were significantly higher in type II diabetic patients (group III) (p-value < 0.05) while significantly lower in group II subjects (p-value<0.05) as compared to controls.

Table-1: Comparison of anthropometric parameters among group I, II and III subjects

Parameters (unit)		Group I n=40	Group II n=40	Group III n=30	p	p_1	p_2	p_3
Age (years) Median (IQR)		32.00 (31.00-36.00)	34.00 (32.00-38.00)	47.50 (44.25-50.00)	0.00*	0.00*	NS	0.00 *
Systolic blood pressure(mmHg) Median (IQR)		120.00 (110.00-125.75)	120 (110.00-120.00)	130.00 (120.00-130.00)	0.00*	0.00*	NS	0.00*
Diastolic blood pressure (mmHg) Median (IQR)		80 (70.50-85.00)	80.00 (78.50-85.00)	80.00 (80.00-80.00)	0.83	NS	NS	NS
BMI (kg/m^2) Median (IQR)	Males	23.96 (21.58-26.14)	25.66 (22.07-27.74)	22.83 (22.65-27.66)	NS	NS	NS	NS
	Females	23.78 (21.11-27.32)	26.36 (22.69-27.64)	22.76 (21.96-25.29)	NS	NS	NS	NS
Waist circumference (cm) Median (IQR)	Males	88.45 (81.67-94.93)	95.25 (86.36-100.52)	90.00 (88.50-100.00)	NS	NS	NS	NS
	Females	78.90 (74.38-89.10)	87.50 (84.00-94.63)	80.00 (79.00-89.00)	0.02*	NS	0.01*	NS

Results are expressed as Median (IQR)
*Statistically significant ($p < 0.05$); compared by Kruskal-Wallis and Mann-Whitney U tests p_1 for group I and III, p_2 for group I and II, p_3 for group II and III. Group I (non-diabetic adult offspring of non-diabetic parents), group II (non-diabetic adult offspring of type II diabetic parents) & group III (type II diabetic patients)

Table-2: Comparison of biochemical parameters among group I, II and III subjects using Kruskal-Wallis and Mann-Whitney U tests

Biochemical Parameters (unit)	Group I n=40	Group II n=40	Group III n=30	p	p_1	p_2	p_3
Fasting serum glucose (mg/dl) Median (IQR)	79.81 (74.85-89.42)	83.97 (78.86-96.62)	102.60 (88.78-137.87)	0.00*	0.00*	NS	0.00*
	81.67± 10.24	85.72± 11.17	121.58± 52.88				
Fasting serum insulin (µIU/ml) Median (IQR)	4.25 (3.43-5.48)	13.45 (11.40-15.88)	14.70 (8.28-18.10)	0.00*	0.00*	0.00*	NS
HOMA-IR Median (IQR)	0.90 (0.64-1.03)	2.73 (2.21-3.79)	4.13 (2.45-5.20)	0.00*	0.00*	0.00*	0.04*
HOMA-%S Median (IQR)	111.11 (97.09-156.25)	36.63 (26.37-45.29)	24.24 (19.26-41.00)	0.00*	0.00*	0.00*	0.04*
HOMA-%B Median (IQR)	70.90 (48.64-117.75)	234.01 (147.48-334.50)	107.01 (65.11-218.62)	0.00*	NS	0.00*	0.00*
Fasting serum visfatin (ng/ml) Median (IQR)	11.25 (9.50-13.00)	9.00 (8.00-10.88)	17.00 (12.38-20.50)	0.00*	0.00*	0.00*	0.00*

*Statistically significant ($p < 0.05$)
Results are expressed as Median (IQR) p_1 for group I and III, p_2 for group I and II, p_3 for group II and III

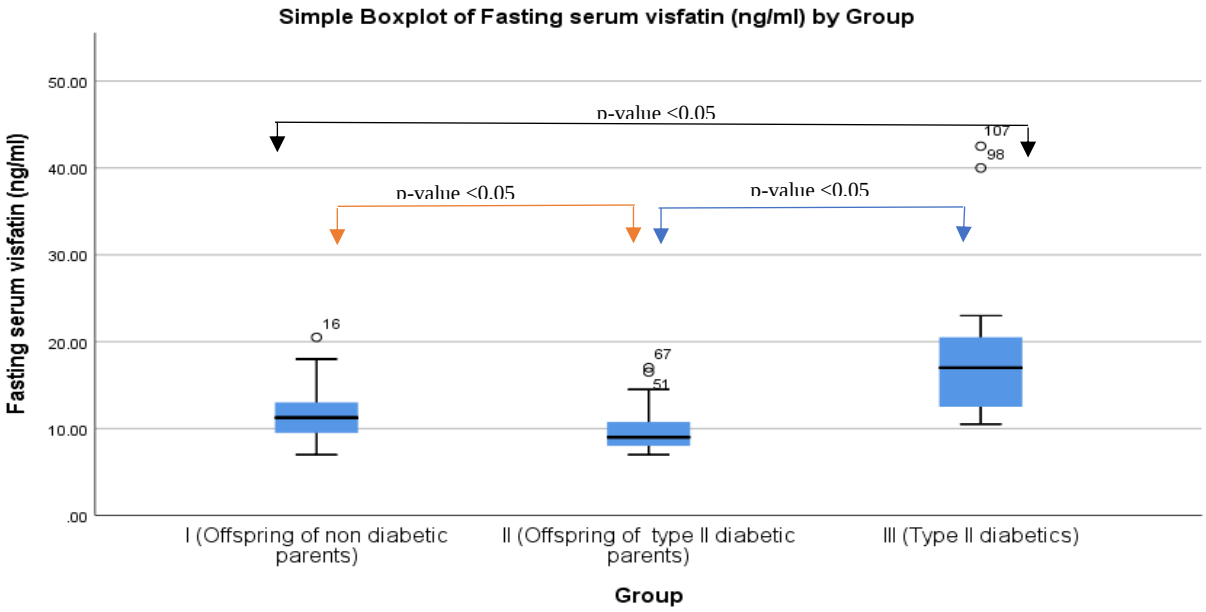

Figure-1: The median and interquartile range (IQR) of fasting serum visfatin (ng/ml) of group I (non-diabetic adult offspring of non-diabetic parents) and group II (non-diabetic adult offspring of type II diabetic parents) and group III (type II diabetic patients) The lower and upper margins of the boxes represent the 25th and 75th percentiles, with the extended arms representing the 10th and 90th percentiles respectively. The median is shown as the horizontal line within the boxes. Outlying points are shown individually.

Fasting glucose levels of group III were significantly elevated than the group II (p-value < 0.05) and the controls (p-value < 0.05) while no significant difference in the glucose levels of group II and controls was observed. Both groups II and III had significantly high fasting insulin levels (p-value < 0.05) and insulin resistance (HOMA-IR) (p-value < 0.05) in comparison to the control group. Insulin sensitivity (HOMA-%S) was significantly lower in both groups II and III than the controls (p-value < 0.05). When groups II and III were compared, it was noted that group III had significantly higher HOMA-IR (p-value < 0.05) and lower HOMA-%S (p-value < 0.05) than group II. Regarding beta-cell function index (HOMA-%β), no significant difference was found between group III and controls. Group II HOMA-%β index were significantly higher than that of control group (p-value < 0.05) and that of group III (p-value < 0.05).

DISCUSSION

In this study, significantly higher visfatin levels were found in type II diabetics (group III) in comparison to the controls (group I). This finding is consistent with the previous studies. Elevated visfatin levels in type II diabetics are suggested by many as a compensatory response to hyperglycemia, impaired tissue signaling or irregular biosynthesis (12, 13). A few have reported low visfatin levels in type II diabetes patients and proposed drug induced enhancement of insulin sensitivity or decreased production of visfatin in the advanced stages as some of the possibilities (14,15). Moreover, as expected, group III had significantly higher insulin resistance (HOMA-IR) and lower insulin sensitivity (HOMA-%S) when compared to the controls. This was in accordance with the previous studies. (16). Beta-cell function index (HOMA-%B) of type II diabetics and the control group had no statistically significant difference while it was significantly lower when compared to group II. Reduction in insulin sensitivity and a decline in beta-cell function (low percentage of HOMA-%β in type II diabetic patients) once the diabetes occurs is supported by the previous literature (17).

On the other hand, low levels of serum visfatin were found in non-diabetic adult offspring with type II diabetic parents (group II) as compared to the controls (group I). Only a few researchers had studied visfatin and related parameters in the non-diabetic individuals with positive family history of type II diabetes (18, 19). Our findings match with them and it seems that non-diabetic subjects with positive parental history of diabetes have low serum visfatin levels. Significantly higher insulin resistance and lower insulin sensitivity were noted in group II when compared to the controls. There is a unanimous agreement on the presence of fasting hyperinsulinemia, high insulin resistance and low insulin sensitivity in individuals genetically predisposed to type II diabetes (20). The percentage of beta-cell function (HOMA-%β) of group II was also higher in comparison to the controls; it probably reflects that the functioning beta cells in group II subjects were hypersecreting in order to maintaining normoglycemia against the increased insulin resistance in the body. This is in accordance with the

previous observations documenting either higher or no significant difference in the HOMA-%β of the non-diabetic adult offspring/first degree relative (FDR) of diabetic patients in comparison to the control group (21,22). Others have noticed lower percentage of beta-cell function in these individuals. (23). Ethnicity, environment and life style changes may account for this disparity in HOMA-beta indices.

Adiposity is a potential source of visfatin (24), a contributory factor for raised insulin resistance (25) and altered beta-cell function (26). In the present study, there was no significant difference in the BMI among three groups. However, group II females had significantly higher waist circumference than the females of the control group. No significant difference in waist circumference of males or females of other groups was obtained. Dysfunctional abdominal adipocytes and insulin resistance are part of type II genetic inheritance. Caloric overload and accumulation of abdominal fat in these individuals leads to lipotoxicity, local and systemic inflammation and further reduction in insulin sensitivity (27).

To summarize, low serum visfatin levels found in non-diabetic insulin resistant subjects with positive parental history of diabetes mellitus in this study could be due to its suppressed production in the body due to hyperinsulinemia. This hyperinsulinemia is genetically as well as due to visceral obesity. Kowalska et al observed a significant fall in circulating visfatin levels in healthy non-diabetic subjects with euglycemic-hyperinsulinemic clamp (28). However, higher visfatin levels in type II diabetics in this study could be explained as a stimulatory effect of chronic hyperglycemia on visfatin production in an insulin resistant environment. Haider and his colleagues supported this finding by showing a rise in circulating visfatin levels with step wise increase in glucose infusions. Co-presence of insulin halted this glucose induced rise (29). A contributory role of chronic low-grade inflammation in the up regulation of visfatin levels in type II diabetic patients has been documented previously (16). Finally, elevated beta-cell function (HOMA-%β) in group II in comparison to control group negates visfatin's role in basal insulin secretion in insulin resistant non-diabetic subjects while higher visfatin levels with non-significant difference in HOMA-%β between group III and controls is a reflection of body's compensation towards raised blood glucose levels and inflammation.

This study has certain limitations including its cross-sectional design and not using the fasting Proinsulin/insulin ratio and clamp techniques for the assessment of the beta-cell function.

CONCLUSIONS

Hyperinsulinemia is most likely responsible for reduction in visfatin levels in in non-diabetic adult offspring of type II diabetic parents and there seems a little role of visfatin in the beta-cell function in the fasting state in these individuals. On the contrary, raised visfatin levels in insulin resistant type II diabetic patients reflect a compensatory response of body to combat hyperglycemia in them.

SOURCE OF FUNDING

This research was conducted at the Post Graduate Medical Institute (PGMI).

CONFLICTS OF INTEREST

None to declare.

ABBREVIATIONS

Indices of insulin resistance (HOMA-IR), insulin sensitivity (HOMA-%S) and beta-cell function (HOMA-% β).

ETHICAL APPROVAL

The study was approved by the Ethical Review Committee of Postgraduate Medical Institute / Ameer-ud-Din Medical College/Lahore General hospital, Lahore

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AUTHOR'S CONTRIBUTIONS

TI: Manuscript Writing
MS: Supervision
SI: Data analysis
ZH: Manuscript Writing (Introduction)
HNL: Manuscript Writing (Methodology)
SN: References writing