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ASSOCIATION OF PLASMA VISFATIN AND OXIDATIVE STRESS MARKERS IN TYPE 2 DIABETES PATIENTS

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Abstract: Metabolic disorder due to type 2 diabetes mellitus in the diabetic patients increases the level of free radicals, which leads to oxidative stress in patients. It affects the insulin secretion and other adipokines, which leads to diabetic complications. The study aims to find out the relationship between plasma Visfatin, and oxidative stress markers in type 2 diabetes mellitus (T2DM) patients. A total of 150 patients with T2DM who had been diagnosed and 150 healthy control participants were enrolled in Index Medical College Hospital and Research Centre, Indore, it was a case-control study and they were matched for age and sex ratio. Glycated HbA1c, glycemic and liver profile, kidney profile as well as certain oxidative stress markers *Lipid peroxidation* (LPO), *catalase* (CAT), *superoxide dismutase* (SOD), *glutathione peroxidase* (GPx), *Glutathione reductase* (GR), *Reduce Glutathione reductase* (GSH), *Protein carbonyl* and *Visfatin* were estimated. The observe results were presented in mean ± SD. Controls and cases were compared using "t-test" and ANOVA. The correlation were determined by the person's correlation "p" value <0.0001considered significant. The data observed shows a clear significance difference between cases and controls in the association of *Visfatin* and oxidative markers .The elevated Visfatin p=<0.0001) values show a contrary down regulation of oxidative stress contour markers such as Catalase, Glutathione, Glutathione peroxidase, Superoxide dismutase and hence depicting that there may be a relation of Visfatin with oxidative stress**.**

Keywords: Visfatin, Oxidative stress biomarkers, diabetes mellitus.

Introduction:

Diabetes Mellitus (DM) is a chronic condition that impacts nearly 6% of the global population, and its prevalence is steadily rising [1]. According to multiple studies performed, the worldwide population of individuals with DM was estimated to approach 221 million by the year 2010, in contrast to the 124 million estimated for 1997 [2-3]. More than 90% of these individuals will have type 2 DM. While there is no unanimous consensus on the precise cause of DM, it is widely acknowledged that the condition has a multifactorial etiology, involving both genetic and environmental factors [4] in which it is found that environmental factors contributes to both type 1 and type 2 and other factors of DM may include immunological responses to various triggers [5] and certain viral infections [6]. In addition Genetic factors, in conjunction with obesity, play a pivotal role in the pathogenesis of type 2 DM. Multiple studies have demonstrated that diabetes mellitus linked to obesity, along with associated metabolic syndrome features like hyperlipidemia

and hypertension, can be attributed to visceral intra-abdominal white adipose tissue [7-10]. Adiponectin, resistin, leptin, adipsin, angiotensin, and estradiol are only a few of the hormones that are produced by adipose tissue [11]. Obesity frequently occurs from a confluence of inadequate exercise, hormonal and genetic factors, and poor eating choices.

Visfatin has been discovered in many tissues and organs, including the brain, kidney, lung, spleen, and testis however it is mostly expressed in visceral adipose tissue, Visfatin may be found in both the cytoplasm and nucleus of cells. [12-13] Visfatin has a variety of purposes as an endocrine, autocrine, and paracrine peptide, including promoting cell proliferation, nicotinamide mono and dinucleotide production, and hypoglycemic effects. [14] Through its effects on glucose metabolism, Visfatin, a recently identified adipokine hormone, shows a clear connection to type 2 diabetes mellitus. [15] Visfatin inhibits the release of glucose from liver cells and increases the uptake of glucose by adipocytes and myocytes, causing hypoglycemia. Visfatin binds to insulin receptors at a location different from that of insulin. [16] Visfatin is upregulated by hypoxia, inflammation, and hyperglycemia, whereas it is downregulated by insulin, somatostatin, and statins. It is mostly found in visceral adipose tissue and has actions similar to insulin. [17] In other hand we have explained that oxidative stress plays an important role in the development of diabetes. Oxidative stress can affect several facts of diabetes, a chronic metabolic illness characterized by increased blood glucose levels. Here are some crucial aspects about how oxidative stress affects diabetes. Reactive oxygen species (ROS) are produced in excess compared to the body's capacity to neutralize those using antioxidants, which causes oxidative stress. Highly reactive chemicals known as ROS, including hydrogen peroxide and superoxide radicals, can harm cellular components and biomolecules, including proteins, lipids, and DNA. [18] Oxidative stress can impair the function of pancreatic beta cells that make insulin. When these cells are exposed to high levels of ROS, their ability to produce and secrete insulin is compromised. This leads to insulin insufficiency and contributes to the development of type 2 diabetes. [19] Oxidative stress is also associated with insulin resistance, which is a hallmark of type 2 diabetes. High levels of ROS can disrupt insulin signaling pathways in insulin-responsive tissues like muscle, liver, and adipose tissue. This impairs the uptake of glucose into cells, leading to elevated blood sugar levels. [20] Oxidative stress can trigger inflammation in various tissues, including adipose tissue. A major contributing element to the emergence of insulin resistance and type 2 diabetes is chronic inflammation. Inflammatory cytokines produced in response to oxidative stress can interfere with insulin signaling. [21] Prolonged exposure to oxidative stress in diabetes can lead to the development of diabetic complications, such as diabetic neuropathy, nephropathy, and retinopathy. Oxidative damage to blood vessels and nerves contributes to the pathogenesis of these complications. [22] The body has natural defense mechanisms to counteract oxidative stress, including enzymatic antioxidants like superoxide dismutase and catalase, as well as non-enzymatic antioxidants like vitamins C and E. However, in diabetes, these defense mechanisms may be overwhelmed, leading to increased oxidative damage. [23] Several lifestyle factors associated with diabetes, such as a high-sugar diet, obesity, and physical inactivity, can contribute to oxidative stress. These factors can increase the production of ROS and decrease antioxidant defenses. [24-31]

Material & Methods:

It was a case-control study, total 300 patients of age 35 and 65 years attending the Medicine OPD's at Index Medical College Hospital and Research Centre, Malwanchal University, Indore (MP). The patients were later divided into two groups' case 150 and control 150, on the basis of diabetes and normal subjects. History, physical examination, and various routine biochemical investigations were done to rule out the subjects.

Inclusion Criteria:

Male and female between 35 and 65 years of age for all groups, screening of T2DM were done as (American Diabetes Association's ADA 2019) diagnostic criteria Subject with fasting plasma

glucose ≥ 126 mg/dl and 2 hours plasma glucose ≥ 200 mg/dl or random blood sugar ≥ 200 mg/dl, HbA1c levels above 6.5% were used to make the diagnosis criteria.

Exclusion Criteria:
Patients with type 1diabetes, pregnant females and Lactating mothers, Patient with impaired blood glucose levels and smokers and alcoholics were not considered to make the diagnosis, Subjects suffering from disease like psychiatric disorder, chronic heart disease, Chronic liver disease, Smokers, Alcoholic, were excluded from the study.

Sample collection:
Clinically diagnosed Diabetic Patients attending the Medicine OPD's at Index Medical College Hospital and Research Centre, Malwanchal University, Indore (MP) were included in the study. A detailed clinical history, including age, sex, occupation, and socio-economic status, will be taken. Subjects will be explained in detail about the study, and written informed consent will be taken. 5.0 ml peripheral blood samples were collected from the patients as well as the controls.

Laboratory Investigation:
Total of 5.0 ml peripheral blood samples was collected from the patients as well as the controls from which 2 ml blood in EDTA vial for HbA1c and plasma/lysate preparation for oxidative stress marker and 1.5 ml blood in plain vial for biochemical parameters (Blood glucose level, Liver function test, kidney function test) by auto-analyzer and 1.5 ml for serum for ELISA estimation of Visfatin.

Assay of Visfatin:
In this procedure circulatory level of Visfatin is quantitative determination of human Visfatin in serum. The serum samples are quantitatively analyzed by sandwich ELISA technique, Human Visfatin GENLISA ^{TM} ELISA kit of KRISGEN Biosystem from USA are used for assessing the Visfatin biomarker in serum samples. Sensitivity of the reaction is defined as the lowest concentration that can be determined with an acceptable and limit of quantification was found to be 8.0 ng/ml.

Plasma and Lysate preparation:
Whole blood is collected in an EDTA vial for the preparation of lysate and plasma. Centrifugation is used to separate the plasma from the blood, In the bottom of the tube, the RBC pellet is intact. The tube will be washed twice with 0.9 percent normal saline at 10,000 revolutions per minute for 15 minutes each. After that, the supernatant is discarded. Finally, chilled triple-distilled water is added to the RBC pellet in proportions equal to the total volume of the whole blood drawn. The sample is now centrifuged at 10,000 rpm for 15 minutes at 4°C, and the pellet and supernatant (Lysate) are collected.

Estimation of Oxidative Stress Biomarkers:
The estimation of *Lipid peroxidation* (LPO), catalase **(CAT)** activity in lysate was determined by the method of Sinha *et al.*,1972.[32], Superoxide dismutase **(SOD)** measurement - SOD activity in lysate was evaluated by the method of Kakkar *et al.*, 1984.[33], Glutathione peroxidase **(Gpx)** measurement - Gpx activity in lysate was evaluated by the method of Rotruck *et al*, 1973.[34] and Estimation of Glutathione **(GSH)** - GSH activity in lysate was determined by the method of Rahman I .*et al*, 2006 [35] Estimation of Glutathione reductase **(GR)** - activity in lysate was determined by the method of Hazelton and Langed *et al*, 1985. [36] Estimation of lipid peroxidation (LPO) activity in plasma will be determined by the method of Stocks and Dormandy *et al*,1971, [37] Protein carbonyl group assay activity in lysate was determined by the method of Levine and Williams *et al*, 1994. [38]

Results:

Diabetic markers Fasting blood sugar, HbA1C, and Postprandial blood sugar in Diabetes Mellitus exhibit substantial increases ($P < 0.05$) in comparison to the control group, as mention in [Table 1] Oxidative stress markers Significant gains ($P < 0.05$) are seen in [Table 3] as, lipid peroxidase (LPO), Catalase (CAT), Superoxide dismutase (SOD), Glutathione Peroxidase (GPx), Reduce Glutathione reductase (GSH) ,Glutathione reductase (GR) Lipid peroxidation(LPO) and Protein carbonyl contrasted with the control group in DM patients.

Statistical analysis:

The acquired data underwent meticulous organization, tabulation, and comprehensive statistical analysis using SPSS statistical software. Qualitative data in this study were represented numerically and as percentages. Quantitative data were presented as mean values along with their corresponding standard deviations (SD). The significance of the findings was determined by evaluating the P-values associated with the respective test statistics. A significance level of 0.05 was adopted, whereas P-values greater than 0.05 were considered statistically insignificant, those equal to or less than 0.05 were deemed significant, and P-values less than 0.001 were classified as highly significant.

Table 1 - Diabetes indicators in diabetic and control groups.

No.	Diabetic Markers	Control (N=150)	T2DM (N=150)	P value
1	**HbA1c (%)**	4.99 ± 0.28	8.21 ± 1.56	0.001
2	**FBS (mg/dl)**	78.76 ± 11.51	202.33 ± 55.10	0.001
3	**PPBS (mg/dl)**	98.87 ± 10.59	277.10 ± 76.45	0.001

Table 2 – Visfatin biomarker in diabetic and control groups.

No.	Diabetic Markers	Control (N=150)	T2DM (N=150)	P value
1	**Visfatin**	10.54 ± 1.53	42.36 ± 4.11	0.0001

Table 3 -Oxidative stress markers in both the diabetes and control groups.

No.	Oxidative stress markers	Control (N=150)	T2DM (N=150)	P value
1	**LPO (nmol MDA/ml)**	2.06 ± 0.87	5.56 ± 1.58	< 0.0001*
2	**SOD (unit/mg protein)**	6.42 ± 2.27	3.02 ± 1.29	< 0.0001*
3	**Catalase (unit/mg protein)**	12.38 ± 1.61	9.94 ± 2.34	< 0.0001*
4	**GR (unit/min/mg protein)**	1.17 ± 0.22	0.94 ± 0.21	< 0.0001*
5	**GPx (nmol NADPH oxidized/min/mg protein)**	49.37 ± 9.91	26.48 ± 4.42	< 0.0001*
6	**GSH umolGSH/mg protein**	3.71 ± 1.01	1.63 ± 0.63	< 0.0001*
7	**Protein Corbonyl (µmol/L)**	0.071 ± 0.02	0.17 ± 0.06	< 0.0001*

The data observed clear significance difference of cases vs control of the prominent markers that is Visfatin elevation (42.36 ± 4.11 vs10.54 ± 1.53 p=<0.0001) and on contratary down regulation of oxidative stress contour markers such as Glutathione (GSH) (3.71 ± 1.01vs 1.63 ± 0.63 p=<0.0001) Glutathione peroxidase **(GPx)** (49.31±9.91 vs 26.48 ± 4.42 p=<0.0001), superoxide dismutase **(SOD)** (52.5±31.5 vs 64.4± 19.6 p=<0.0001) and Catalase **(CAT)** (12.38 ± 1.61 vs 9.94 ± 2.4 p= <0.0001), LPO (2.06 ± 0.87 vs 5.56 ± 1.58 p=<0.0001) depicting a clear association of Visfatin can be a contributor of diabetes and also down regulation of oxidative contour markers can causes increase in oxidative stress and can worsen the condition and also from the above mentioned one can assumed that there can association of Visfatin in down regulating oxidative stress controlling markers.

Discussion:

Type 2 diabetes (T2DM) poses a serious threat to world health, accompanied by an elevated risk of cardiovascular issues and severe chronic complications, including retinopathy, nephropathy, and neuropathy, which contribute to increased morbidity and mortality. It was also found that Reactive oxygen species may play a central role in the pathophysiology of Type 2 diabetes mellitus.[39-40] As, oxidative stress, is characterized by an imbalance between oxidants and antioxidants, it can be assessed using the oxidative stress index (OSI), which is the ratio of total oxidant status (TOS) to total antioxidant status (TAS). The study included Visfatin an adipokine which is a 52 kDa protein produced by visceral adipose tissue, is known to stimulate cytokine production, and is recognized as a pro-inflammatory adipokine. [41-42] Clinical studies have established links between Visfatin and conditions like Diabetes mellitus, acute ischemic stroke, acute pancreatitis, and myocardial infarction. While Visfatin has been generally associated with adverse long-term outcomes, it has also demonstrated regulatory effects in myocardial, neuronal, and mitochondrial contexts. However, further investigations are needed to precisely define its impact on critically ill patients. [43]

The present study aimed to explore the relationship between Visfatin and oxidative stress [44] in diabetes mellitus, considering numerous research findings suggesting that reactive oxygen species (ROS) play a pivotal role in exacerbating hyperglycemia and inducing oxidative stress in diabetes, this is done by evaluating blood glucose levels, Visfatin, as well as the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, along with monitoring glutathione levels.

The results of the study indicated a significant increase in Visfatin levels in diabetic patients compared to controls, while CAT activity was significantly decreased in T2DM patients compared to controls. A similar trend was observed for SOD activity, glutathione, and glutathione peroxidase levels in T2DM patients. The strong association between elevated Visfatin levels and low levels of oxidative stress-controlling markers, such as CAT, suggests that increased Visfatin may exacerbate oxidative stress and worsen the diabetic condition; hence oxidative stress is closely intertwined with the development and complications of diabetes. Mitigating oxidative stress through lifestyle modifications and potential antioxidant therapies could potentially have a positive impact on diabetes management and the prevention of related complications.

Conclusion:

According to the obtained results of the diabetic mellitus patients and controls the findings of the present investigation revealed that diabetic patients experience higher levels of oxidative stress, and raised level of Visfatin in diabetic patients indicates the sever diabetic problem in future, and it is strongly correlated in T2DM and it also been concluded that increased oxidative stress with elevated Visfatin levels and both conjointly can worsen the condition of type 2 mellitus patients.

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