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Genetic Association of Visfatin Gene G-948T Polymorphism with Biochemical Parameters and Oxidative Stress Markers in Type 2 Diabetic Patients

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KEYWORDS

Visfatin; Blood glucose; Diabetes mellitus; Hyperglycemia; Hyperlipidemia.

ABSTRACT:

Visfatin are bioactive molecules that control a wide range of physiological processes, including insulin action, the inflammatory process, and vascular homeostasis. The aim of this study is to ascertain the relationship between serum visfatin levels and other biochemical markers. More than 300 people from Outpatient Department of Medicine of Santosh Medical College took part in the study. Out of the 300 participants chosen, 150 had type 2 diabetes, while the remaining 150 acted as the control group. All subjects' lipid profiles, fasting plasma glucose, glycosylated hemoglobin, and visfatin serum concentrations were examined. The outcome showed that the visfatin serum concentration were higher in subjects with type 2 diabetes mellitus than the control groups. Visfatin was found to be correlated with some biochemical parameters. From the obtained results we concluded that the assessment of visfatin levels and their relation to some metabolic parameters can help to identify subjects who are more susceptible to cardiovascular disease.

1. Introduction:

High blood glucose levels are a hallmark of diabetes mellitus, a group of metabolic disorders brought on by flaws in insulin function, production, or both (Garcia *et al.,* 2020). In India, T2DM is on the verge of becoming a pandemic illness. Obesity, dyslipidemia, stress, hyperglycemia, and physical inactivity are all prevalent risk factors for both T2DM and coronary artery disease (Mohan *et al.,* 2010). Adipokines are hormone-like proteins or peptides that are secreted by adipose tissue. By regulating insulin sensitivity, energy homeostasis, and fat metabolism, several adipokines, including omentin-1, visfatin, leptin (a protein released by fat cells), resistin, and adiponectin, have a substantial impact on metabolic illnesses linked to obesity (Balistreri *et al.,* 2010). Adipokines alter the function of endothelial cells, arterial smooth muscles, and macrophage in vessel walls, which links fat with atherosclerosis (Yoo *et al.,* 2014).

A novel adipocytokine called visfatin has recently been shown to be related with visceral fat in both mice and humans (Romacho *et al.,* 2013). Visfatin was originally isolated from peripheral blood cells as a secreted growth factor that promotes B-cell precursor maturation (Romacho *et al.,* 2013). When questions were raised about visfatin's ability to replicate the effects of insulin by binding to the insulin receptor and lowering plasma glucose levels, this research was eventually retracted. However, the identification of this adipokine holds considerable promise for improving knowledge of the aetiology of obesity (Abdalla, 2022). Numerous researchers have looked at the function of visfatin in disorders connected to diabetes and obesity, regardless of how it works. Visfatin's pathophysiological function in humans is yet unknown. The objective of the current study was to look into the function of visfatin in type 2 diabetes mellitus with regard to biochemical parameters because there isn't

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much agreement on the normal visfatin concentration and its link to metabolic parameters in adults. Our data demonstrate that elevated serum visfatin in type 2 diabetes is related to biochemical factors as a whole.

2. Material and Methods

2.1 Chemicals and glassware

Sigma-Aldrich in the United States and Hi-Media Laboratories Pvt. Ltd. in Bombay, India provided the chemicals employed in the current study. Coral Clinical Systems (Goa, India) provided the kits used for biochemical estimation, while Borosil Glass Works Ltd. (Mumbai, India) provided the glassware required for experimental work.

2.2 Study design and participant subjects

This study was conducted in the department of Biochemistry at the Santosh medical college. An observational case-control study was conducted. The current study included more than 300 participants from Santosh Medical College's Outpatient Department of Medicine. Out of the 300 participants chosen, 150 had type 2 diabetes, while the remaining 150 acted as the control group. The American Diabetes Association (ADA, 2022)'s criteria were used to determine the diagnosis of T2DM in each subject. After receiving proper approval from the institutional ethical committee, the study got underway. Prior to the event, each participant got counseling about diabetes, including its causes, symptoms, complications, etc. Before registering, all subjects were made aware of the study's goals. Before giving their written agreement, every subject was fully informed of the advantages and disadvantages of the study.

Inclusion and exclusion criteria employed for selection of type II diabetic subjects:

Inclusion Criteria

(1)Males and females with type 2 diabetes mellitus in the age group of 35 to 75 years. (2) Subjects having body mass index (BMI) between 18.5 and 40. (3) Elevated blood glucose levels (fasting blood glucose ≥ 126 mg/dl and postprandial blood glucose ≥ 200 mg/dl). (4) Participants in the study must be willing to follow the procedure and give written informed consent.

Exclusion criteria:

(1)Those with any kind of chronic illness or diseases or disorders (2) pregnant females and lactating women were excluded from the study. (3) Those on steroid therapy for other ailments. (4) Those addicted to alcohol or other drugs.

2.3 Collection and processing of blood samples

After a 10 to 12-hour overnight fast, blood samples were taken from each individual. The estimation of the glucose level was done using plasma that had been isolated. Fresh whole blood samples of 0.1 ml were carefully mixed with 0.9 ml of distilled water to determine the amount of glycosylated hemoglobin. The remaining sample was refrigerated at -20℃ until it was utilized to estimate other biochemical parameters.

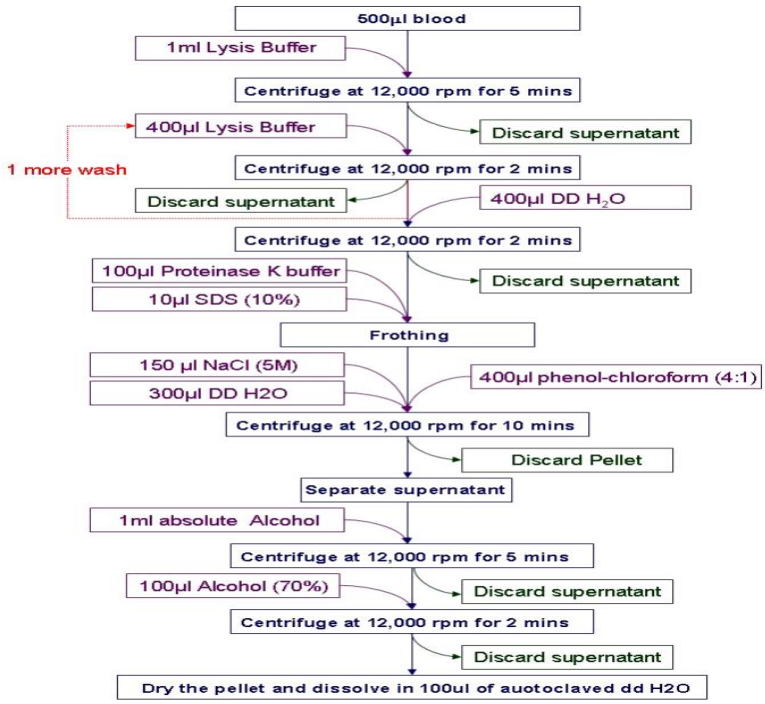

Figure: Flow diagram of DNA isolation protocol

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2.4 Biochemical parameters

Estimates were made for the lipid profile, HbA1c, fasting and postprandial plasma glucose, and glycosylated hemoglobin (HbA1c). Raobdo and Terkildsen (1960) used the glucose oxidase/peroxidase method to estimate fasting and postprandial plasma glucose. Trivelli *et al.* (1971) used the ion exchange resin method to determine the amount of glycosylated hemoglobin (HbA1c). With the help of standard kits from Crest, plasma total cholesterol was estimated using the Cholesterol Oxidase Phenolaminophenazone CHOD-PAP method (Stockbridge *et al.*, 1989), triglycerides using the GPO-PAP method (Fossati and Prencipe, 1982), HDL-C using the Polyethyleneglycol/Cholesterol Oxidase-Phenol-aminophenazone method (Lopes-Virella *et al.*, 1977), using standard kits from Crest Biosystems, Goa (India), Low-density lipoprotein cholesterol (LDL) and Very-low density lipoprotein cholesterol (VLDL) were computed using the Friedewald's formula. Human visfatin ELISA kit (Bio Vision, Mountain View, CA, USA) measurements of serum visfatin concentrations were done in duplicate using a microplate reader.

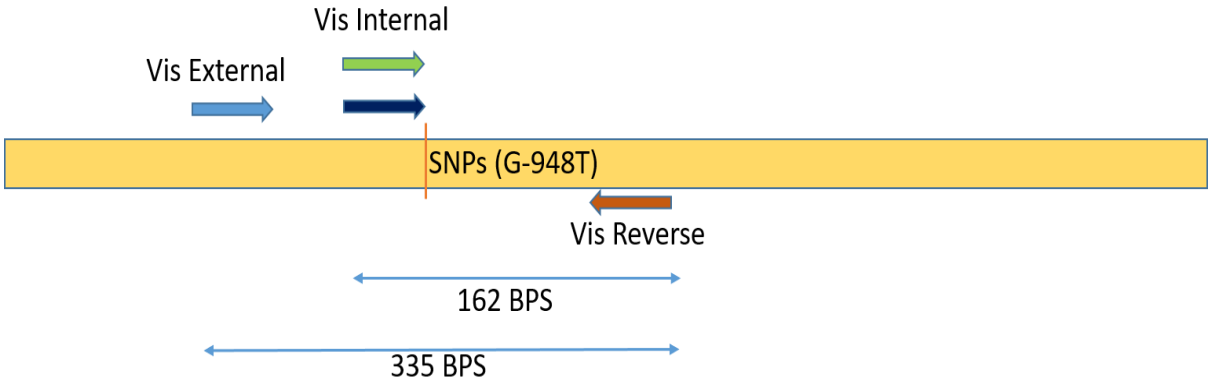
2.5 Genetic polymorphism Analysis

The polymorphic genotyping was determined by using polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and Agarose Gel Electrophoresis Techniques.

The genomic DNA samples were amplified by polymerase chain reaction (PCR). For PCR amplification a forward primer 5'-CGTGGCCCTGTGGCAGCCGA–3' and reverse primer 5' GGGCTCGTTAGGAGCTGAGGG-3' were applied. A 424 bp DNA fragment of HNF-1α gene was obtained on 2% agarose gel electrophoresis. To PCR product was generated in the final volume of 11 µl containing 1µl genomic DNA, 5.4µl PCR master mix, primers and 4.24 µl H_2O was used to obtain PCR products. The PCR protocol was: 95^0 C for 4 min followed by 35 cycles of 95^0 C for 45 sec, 62^0C for 35secs 72^0 C for 42secs and final extension at 72^0 C for 7 min. The PCR products were digested with 2.5 U of *HaeIII* restriction enzyme and electrophoresed on a 3.5% agarose gel. Finally the *HaeIII* Restriction Fragment Length Polymorphism (RFLP) was detected by ethidium bromide staining.

The SNPs (G-948T) of visfatin gene were examined by real-time PCR for melting curve analysis on a LightCycler after DNA separation. We used 20µl of reaction mixture containing 5µl of DNA sample, 0.5µlM of each primer, 0.2 µlM of each probe, and 3mM $MgCl_2$. PCR conditions and melting curve parameters were optimized. The primer sequences are shown below.

VISFATIN F (I)	AGCCTTTGACAGGGTGCGAC
VISFATIN F1(I)	AGCCTTTGACAGGGTGCGAA
VISFATIN F (E)	CCGGCGGCTCTGTCTATGG
VISFATIN R	GCAACCACGCATGAGAACTGC



2.6 Statistical analysis:

Statistical Package for Social Science (SPSS, Chicago, IL, USA) version 20.0 and Excel (Microsoft 13) were used for the data analysis. The results were expressed as Mean ± Standard Deviation.The statistical differences between cases and control were determined by student independent sample t-test. Pearson's correlation was used to evaluate the relationship between plasma levels of visfatin and biochemical markers in type 2 diabetes subjects. The p-value < 0.05 was considered statistically significant.

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3. Results

3.1 Participant subjects

A total of three hundred subjects belonging to different groups (Normal healthy subjects, N=150; T2DM= 150) were recruited to the study. The two groups were comparable on entry into the study with respect to age, duration and other clinical and biomedical measures. Although there were differences in some baseline characteristics, these differences did not reach statistical significance. The demographic details of study subjects are given in (Table 1).

Table 1: Baseline characteristics of subjects recruited to the study

Parameters (n=150)(n=150)	Variables	Normal	Diabetic
Mean age(years)	30-70	48	49
Sex (No.)	M/F	105/45	100/50
BMI (Kg/m^2)18-30		24.5±0.8	25.1±0.7
Duration of diabetes	≤ 1	-	85
(Years)	≤ 5	-	65
Systolic blood pressure (mmHg)		127.9±3.2	130.5±3.3
Diastolic blood pressure (mmHg)		78.4±1.4	79.3±1.0

3.2 Overall status of physiological parameter, sugar and lipid profile, oxidative stress biomarkers in subjects with type 2 diabetes mellitus (*N*=150)

Table 2: Overall status of physiological parameter, sugar and lipid profile in Type 2 Diabetes Subjects (*N*=150)

Parameters	Mean ± SD	Minimum	Maximum
BMI	25.1±5.3	14.7	38.3
FBS	176.8±66.5	80.0	439.0
PP	258.9±97.2	94.0	580.0
HbA1C	9.24±2.8	4.7	16.1
TC	203.5±73.6	92.0	405.0
TG	156.4±42.0	60.0	266.0
HDL	38.2±10.7	20.0	68.0
LDL	133.9±73.7	33.4	341.0
VLDL	31.2±8.4	12.0	53.20

Table 3: Overall status of physiological parameter, sugar and lipid profile in Control Subjects (*N*=150)

Parameters	Mean ± SD	Minimum	Maximum
BMI	24.5±3.9	17.9	37.6
FBS	88.4±10.7	61.0	116.0
PP	123.7±14.0	95.0	166.0
HbA1C	5.3±0.5	2.4	6.4
TC	162.3± 32.3	102.0	266.4
TG	128.5±36.8	39.0	236.2
HDL	44.5±8.4	26.9	68.0
LDL	92.1±32.1	26.8	190.2
VLDL	25.7±7.3	7.8	47.2

Table 2&3 shows the average ± SD blood glucose profile of normal group and diabetic group. It is evident from the data that blood glucose of normal control group remains within normal limits whereas diabetic group showed hyperglycemia. The percentage elevation in blood glucose level was 56.2% in fasting blood glucose level (88.4±10.7 to176.8±66.5).The percentage elevation in blood glucose level was 53.8% in postprandial blood glucose level. Moreover, Table 2/3 depicts the level of plasma cholesterol, HDL cholesterol and triglycerides in normal and diabetic group. Cholesterol and triglycerides were marginally elevated (162.3± 32.3mg/dl to 203.5±73.6mg/dl and 128.5±36.8 to156.4±42.0 respectively) whereas HDL-C level decline in diabetic group (44.5±8.4mg/dl to 38.2±10.7 mg/dl).

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Table 4: Overall status of Oxidative Stress Markers in Type 2 Diabetes Subjects (*N*=150)

Parameters	Mean ± SD	Minimum	Maximum
CAT	11.4±3.4	4.6	19.8
SOD	3.7±2.0	0.56	12.0
GR	1.1±0.3	0.32	2.3
GPx	29.0±4.9	11.2	45.2
MDA	5.2±1.7	1.08	9.7
GSH	1.80±0.8	0.52	5.3
PCA	0.20±0.0	0.05	0.39

Table 5: Overall status of Oxidative Stress Markers in Control Subjects (*N*=150)

Parameters	Mean ± SD	Minimum	Maximum
CAT	12.3±1.6	9.21	16.7
SOD	6.32±2.6	2.78	16.7
GR	1.13±0.2	0.64	1.87
GPx	48.2±10.3	23.7	79.8
MDA	2.4±1.0	0.51	4.39
GSH	3.46±1.0	1.44	9.62
PCA	0.07±0.0	0.03	0.14

Oxidative stress results from an imbalance between radical-generating and radical scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both. Diabetes mellitus is accompanied by increased formation of free radicals and decreased antioxidant capacity, leading to oxidative damage of cell components.

MDA has been recognized as a most important biomarker of free radical mediated lipid damage and oxidative stress. Marginally high levels of thiobarbituric acid reactive substances (TBARS) (2.4±1.0nmol MDA/mlto 5.2±1.7nmol MDA/ml) and reduced antioxidant enzyme activities were found in diabetic subjects as compared to normal healthy subject's; catalase (12.3±1.6unit/ml to 11.4±3.4unit/ml); SOD (6.32±2.6unit/mg protein to 3.7±2.0unit/mg protein); GSH (3.46±1.0µmolGSH/mg protein to 1.80±0.8µmolGSH/mg protein); GR (1.13±0.2unit/min/mg protein to 1.1±0.3unit/min/mg protein); GPx (48.2±10.3nmol NADPH oxidized/min/mg protein to 29.0±4.9nmol NADPH oxidized/min/mg protein).

3.3: Comparison of genotype of Visfatin gene between Type 2 Diabetes Subjects and control group*(N=150)*

TABLE 6: Comparison of genotype of Visfatin gene between Type 2 Diabetes Subjects and control group*(N=150)*

Genotype	Type 2 Diabetes Subjects (150) *N* (%)	Control (150) *N* (%)	Chi Square P value
GG	31 (20.7%)	85 (56.7%)	0.000
GT	43 (28.7%)	27 (18%)	
TT	76 (50.7%)	38 (25.3%)	

Table 7: Comparison of mean values of Visfatin genes between Type 2 Diabetes Subjects and control group

Parameters	Type 2 Diabetes Subjects (*N*=150) (Mean±SD)	Control (*N*=150) (Mean±SD)	Significant *P* value
Visfatin	58.70±14.7	23.2±3.7	0.000

The visfatin level of diabetic control group was significantly increased as compared with the controls ($P < 0.05$). Visfatin levels were significantly elevated (23.2±3.7to 58.70±14.7 and respectively) in diabetic group. The changes of plasma visfatin levels in T2DM may also suggest the role of visfatin in the pathogenesis of T2DM

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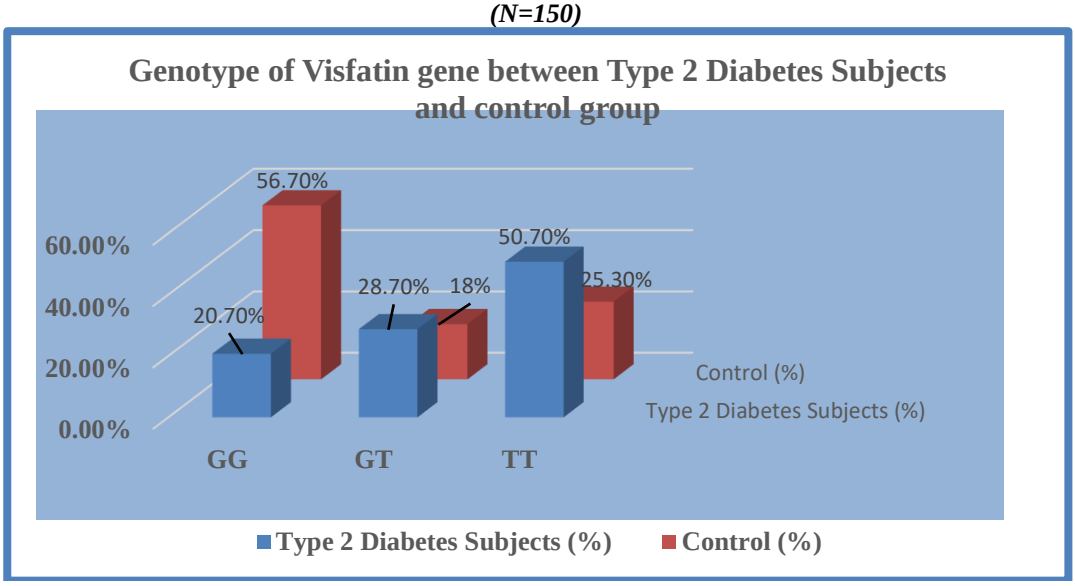
Graph showing comparison of genotype of Visfatin gene between Type 2 Diabetes Subjects and control group *(N=150)*



The visfatin level of diabetic control group was significantly increased as compared with the controls (P <0.05). Visfatin were marginally elevated (23.2±3.7 to 58.70±14.7) in diabetic group. The changes of plasma visfatin levels in T2DM may also suggest the role of visfatin in the pathogenesis of T2DM (Table 7).

4. Discussion

Diabetes mellitus is a chronic, lifelong disease that is today recognized as a major global health problem. Non-insulin dependent diabetic mellitus (NIDDM) is on the rise as a result of acculturation to a western way of life (Richter *et al.,* 2018). The prevalence of NIDDM is rising exponentially (Choudhary and Rajeswari, 2021). The current study aims to evaluate the serum visfatin levels in T2DM patients. The study's findings show that subjects with type 2 diabetes mellitus had serum visfatin levels that were higher than those of healthy people.

Additionally, diabetic dyslipidemia, which is usually present in people with diabetes, is one of the major risk factors for cardiovascular disease (Warraich and Rana, 2017). According to various researches, patients with type 2 diabetes also have low levels of high density lipoprotein (HDL-C) in addition to high levels of cholesterol and triglycerides (Daniel *et al.,* 2019). Our findings corroborate past research that found abnormally increased lipoprotein levels and lower HDL cholesterol levels in both male and female patients with type 2 diabetes mellitus (Beesbrock *et al.,* 1982; Howard *et al.,* 1984; Mattock *et al.,* 1982; Walden *et al.,* 1984). The environmental influences were essentially the same across all study groups. Therefore, the reduced levels of HDL cholesterol lipoprotein in the diabetes group were not due to environmental variables. Triglycerides and HDL cholesterol were compared in all categories and in both sexes, and it was found that there was a constant inverse relationship between the two. The levels of glucose and glycosylated hemoglobin were also measured. According to Rahman et al. (2021), postprandial hyperglycemia is caused by a decline in the absolute level of plasma insulin or action as opposed to a decline in the insulin-to-glucagon ratio, which causes the liver to produce more glucose (basal hyperglycemia). Additionally, we found in our research that type 2 diabetes mellitus patients have significantly increased visfatin plasma levels. An earlier study (Fukuhara *et al.,* 2005) found a correlation between plasma visfatin and insulin resistance. Visceral fat significantly expresses visfatin, a novel adipocytokine that was identified by Fukuhara et al. in 2005 (Fukuhara *et al.,* 2005). According to Samiha *et al.* (2013), visfatin is an adipokine with insulin-like properties that increases muscle and adipocyte glucose transfer while inhibiting hepatocyte glucose synthesis. Furthermore, they claimed that plasma visfatin and the amount of visceral fat measured by computed tomography in human volunteers are strongly correlated (Fukuhara *et al.,* 2005). In a previous study, patients with type 2 diabetes mellitus showed increased levels of visfatin (Chen *et al.,* 2006). Haider et al. found that high blood glucose caused an increase in plasma visfatin by utilizing the glucose clamp test on healthy volunteers (Haider *et al.,* 2006). The association between visfatin and glucose levels was likewise positive, which is relevant for the aetiology of type 2 diabetes mellitus. Based on the aforementioned, our results might support the hypothesis that type 2 diabetes mellitus patients' increased plasma visfatin

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levels are a result of their environment's hyperglycemia. It is well-known that one of the most significant effects of contemporary lifestyles, which consistently increase the risk for the onset of numerous diseases, is obesity. Compared to healthy people, diabetic patients have increased serum visfatin levels. Fasting glucose levels are the primary determinant of elevated visfatin levels in subjects with type 2 diabetes mellitus (Esteghamati *et al.,* 2011). According to earlier research (Dogru *et al.,* 2007; Samiha *et al.,* 2013), visfatin levels were greater in patients with type 2 diabetes mellitus compared to controls. According to this study, visfatin did not connect with BMI in the type 2 diabetes mellitus group but did positively correlate with fasting blood glucose levels and HbA1c. According to Matsuzawa (2006), one of the primary processes in these lifestyle-related diseases is the release of many adipokines by adipose tissue. According to Ahmed et al.'s research, visfain may be involved in the pathophysiology of diabetes and is crucial for the emergence of the metabolic syndrome (Ahmed *et al.,* 2015). The fact that visfatin concentrations are higher in those with type 2 diabetes mellitus may support the theory that visfatin is connected to impaired glucose metabolism. We believe that these data, despite the experimental and methodological constraints, provide important information for the study of visfatin.

5. Conclusion

We deduce from the findings that visfatin may have a role in the emergence of insulin resistance and diabetes mellitus. Additionally, the evaluation of visfatin levels and their relationships with some metabolic syndrome markers can aid in locating individuals who are most at risk for CVD.

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