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Study of Nitro tyrosine and its association with glycosylated haemoglobin in Type 2 diabetic patients

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ABSTRACT
In this case, the person has diabetes mellitus. (T2DM) is a major non-communicable illness and is connected with micro and macro-vascular problems, as well as an increased risk of death. T2DM is caused by a combination of several factors that contribute to this risk, including decreased glucose tolerance, insulin resistance, and β-cell dysfunction, chronic oxidative stress, and dyslipidemia. To investigate the relationship between nitro tyrosine levels and HbA1c, insulin resistance, and lipid profile indicators in T2DM patients and healthy controls. Thirty type 2 diabetic patients aged 35-45 were chosen as subjects, while thirty healthy volunteers aged 35-45 were chosen as controls. The levels of plasma nitro tyrosine and insulin were determined using an ELISA, the level of glycosylated hemoglobin (HbA1C) was determined using an HPLC method, and Other regular studies used an ERBA EM-360 fully automated analyzer.The study found that type 2 diabetics had much higher levels of nitro tyrosine than healthy volunteers. Plasma nitro tyrosine levels were found to be favorably linked with fasting plasma glucose, hemoglobin A1c, and HOMA-IR, UACR, triglycerides, and LDL cholesterol levels. An important oxidative stress marker for type 2 diabetics, plasma nitro tyrosine, has been discovered. As a result, nitro tyrosine may be used as a routine diagnostic marker in people with T2 DM.
Keywords: *T2DM, HbA1C, insulin resistance, homeostasis model (HOMA-IR)*

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INTRODUCTION

Diabetes is a chronic metabolic illness that affects millions of people worldwide. Chronic hyperglycemia and other metabolic abnormalities in diabetic individuals might affect numerous organs, leading to potentially fatal micro vascular and macro vascular consequences (1,2).In addition to persistent hyperglycemia, decreased insulin-mediated vasodilation and increased inflammatory pathways contribute to insulin resistance (3).Diabetes complications are exacerbated by poor glycemic control [6].Chronic hyperglycemia is linked to micro vascular issues [7–9].Improved glycemic control lowers the risk of micro vascular complications in type 2 diabetics [9, 10].In fact, the relationship between GHb, macro vascular problems, and death remains unclear. Some cardiovascular outcomes were better with aggressive glycemic treatment, but not the chance of dying from CVD or any other cause. There were three meta-analyses that found this. Because of low event rates or high rates of not following up, these trials may not have been able to show that they worked. A meta-analysis of ten planned studies found that every 1% rise in GHb caused CVD to happen 18% more often [15].The risk of death from any cause was not assessed in this meta-analysis. Only two of the studies on this list had baseline sample sizes of over 2,000 [16, 17].Recent research on how GHb levels affect CVD outcomes and/or all-cause mortality in type 2 diabetics has yielded inconsistent results. These cohort studies found a U-shape [18] and a non-linear positive correlation [19] for all-cause mortality. Most studies found a favorable CVD outcome correlation [19–24].

It happens when your body is under a lot of oxidative stress that there are more respiratory nitrogen species (RNS) in your body, like hydroxyl radicals and superoxide. Glucose autoxidation, redox potential shifts, diminished antioxidants like glutathione (GSH), vitamin E, and reduced antioxidant defence mechanisms. Oxidative stress in diabetes is caused by SOD, CAT, and glutathione peroxidase [6].Despite this, the antioxidant system doesn't work well, and gene changes make it more likely that pro-oxidant

species will be made. Endogenous change molecules due to Oxidative stress markers include reactive oxygen and nitrogen species in the cellular microenvironment. Protein-bound and free tyrosine residues are nitrated by peroxynitrite molecules [7].Nitro tyrosine (NT) is susceptible to release by different mediators and reactive nitrogen intermediates to create nitro tyrosine (NT) [8,9].The current study's goal was to examine nitro tyrosine levels between healthy people and type 2 diabetics, also glycosylated hemoglobin and insulin resistance.

MATERIAL AND METHODS

Our study included 30 T2DM patients aged 35–45 years from the NIMRA Medical College in Jupudi, Andhra Pradesh. Patients with diabetes, hypothyroidism, or thyroid disease were excluded. thyroid hormone replacement therapy, or a history of nephrotic syndrome, nephrotic syndrome-like illness, or a history of MI, stroke, or OPD.The study used 30 healthy volunteers of the same age as the study's controls and all subjects gave informed consent (IHEC).The experiments followed the Helsinki declaration of 1975.

Biochemical analysis:

Ten minutes after the individuals had fasted, their 10 minutes centrifuged blood at 2000g, the ERBA EM-360 system was used to analyses samples for glucose and lipid profiles (total cholesterol, HDL, LDL, triglycerides). An Enzyme Linked Immune Sorbent Assay was used to assess plasma nitro tyrosine and insulin levels (ELISA).Analysis of urine samples for micro albumin, creatinine, and 2 hours postprandial venous blood for plasma glucose (PPG) analysis was carried out using an auto analyzer. Based on fasting plasma insulin and The HOMA-IR (homeostasis model analysis for insulin resistance) was determined [10].

Statistical analysis:

The data were statistically analyzed in SPSS 25.0.The mean standard deviation was used. by the t-test, and the statistical significance was well-considered. Correlation analysis relied on the Pearson correlation test as a dumping ground for old scores from other tests.

RESULTS AND DISCUSSIONS

Table 1: Baseline parameter comparison: Age, BMI, WHR, blood pressure in T2DM patients and healthy people.

Parameters	N=30 Controllers.	T2 DM patients (n=30)	P -value
Age	38.9±3.2	39.6±.4.3	0.07
BMI	24.2±1.5	29.8±1.3	0.04#
Waist/Hip ratio	0.92±0.05	0.95±0.07	0.03#
BP (mm Hg)	112.4±4.4	131.2±5.5	0.01*
Diastolic (mm Hg)	75.4±3.9	89.1±4.1	0.01*

The data is shown as the presented as mean and standard deviation (mean SD). *p0.001 and #p0.05 were statistically significant in a well-thought-out way.

Table 2: Fasting and postprandial glucose comparison HOMA-IR, urinary albumin creatinine ratio, plasma nitro tyrosine markers in T2 DM patients and controls

Parameters	**N=30 Controllers.**	**T2 DM patients (n=30)**	**P value**
FPG(mg/dl)	85.5±9.8	143.7±7.7	0.001*
PPG (mg/dl)	107.3±8.6	187.1±10.4	0.001*
Total cholesterol	169.5±10.2	193.5±16.9	0.04#
Triglycerides (mg/dl)	108.6±15.2	182.8±16.8	0.03#
HDL (mg/dl)	45.1±6.4	40.6±5.8	0.03#
LDL (mg/dl)	118.6±12.2	155.8±16.7	0.001*
HbA1c	5.7±0.8	9.3±1.5	0.001*
Insulin (µ IU/ml)	7.6±1.1	12.8±3.2	0.001*
HOMA –IR	1.4±0.14	4.9±2.1	0.001*
Urinary albumin creatinine ratio (mg/gm Creatinine)	21.3±2.8	78.8±12.5	0.001*
Plasma Nitro tyrosine (µmol/l)	0.31±0.06	0.57±0.12	0.001*

The data is shown as the presented as mean and standard deviation (mean SD). *p0.001 and #p0.05 were statistically significant in a well-thought-out way.

Table 3: Correlation between Plasma Nitro tyrosine &measured parameters in study subjects

Parameters	Coefficient-r	P -value
FPG	0.365	0.04*
PPG	0.489	0.07
HbA1c	0.398	0.02*
HOMA-IR	0.421	0.01**
UACR	0.393	0.01**
Cholesterol	0.198	0.06
TGL	0.421	0.04*
HDL	-0.297	0.07
LDL	0.367	0.04*
BMI	0.145	0.08
Waist /Hip ratio	0.124	0.07

*The correlation is significant at 0.05 (2-tailed).*The correlation is 0.01 (2-tailed) (2-tailed).

DISCUSSION

In type 2 diabetics, Oxidative stress contributes to vascular problems. Reactive nitrogen and carbonyl species (RNS) slow down oxidation in the cell (RCS), which all play a role in oxidation [11, 13].Inflammation, age, drug interactions, and toxicity can all lead to an imbalance between antioxidants and pro-oxidants, which can lead to cell death [14-16].According to the results of this study, T2DM patients have a higher BMI and a waist-to-hip ratio than healthy volunteers. Adipose tissue has an innate immune system that produces pro-inflammatory cytokines and causes oxidative stress. This makes people fat and causes them to have a lot of chronic systemic inflammation. According to a number of studies [17–19], adipose tissue inflammation has been linked to the development of obesity-related problems in type 2 diabetes patients. T2DM patients had greater total, LDL, and HDL cholesterol than healthy adults. In diabetic patients, changes in lipid parameters are linked to an increase in the free fatty acid flux pool and improved insulin sensitivity (20).According to the current research, a considerable difference was found in systolic and diastolic blood pressure between people with diabetes and healthy volunteers. Twenty-one. Insulin resistance can cause problems with membrane ion transport and growth of vascular smooth muscle cells, all of which raise the risk of high blood pressure [21]. Increased plasma nitro tyrosine levels in patients with type 2 diabetes (T2DM) compared to healthy volunteers, as well as positive correlations with FPG, Hb A1c, HOMA-IR, UACR, triglycerides, and LDL cholesterol levels were found in this study. Superoxide anion and nitric oxide imbalances cause alterations in vascular function that stimulate the formation of peroxynitrite [22]. Nitro tyrosine is made when peroxynitrite is used to nitrate proteins' tyrosine residues [23]. As nitro tyrosine concentrations rose in a wide range of diseases, the function of endothelial cells, the relaxation of blood vessels, and the damage to DNA in endothelial cells were all affected [24, 25].In the body, ROS and NO react with each other, forming cytotoxic reactive nitrogen species (RNS) that can damage blood vessels if they are present for a long time [26, 27]. If this happens, proteins and other molecules that harm the vascular system can be nitrated. Endothelial abnormalities may have higher nitro tyrosine peroxynitrite in hyperglycemia. The group may need this. The nitrosylation of VLDL has been linked to atherosclerosis and altered lipid transport in the body. Reduced antioxidant defense mechanisms, peroxynitrite, and nitrosylated VLDL peroxidation all contribute to coronary vascular disease complications [29].

CONCLUSION

As a result, plasma nitro tyrosine is a great way to tell if someone has type 2 diabetes. The use of nitro tyrosine as a diagnostic marker in T2DM could be carefully thought out to reduce cardiovascular morbidity and death. As a result, we found that individuals with type 2 diabetes who had persistent hyperglycemia had a higher risk of cardiovascular disease and death. This danger is distinct from other known risks. A higher GHb level in diabetics increases the risk of heart disease and death. As a result of our findings, it may be beneficial to aim for GHb levels that are as close to normal as possible.

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