THE ASSOCIATION BETWEEN SERUM TRIGLYCERIDES, SERUM HNF1ALPHA, AND OXIDATIVE STRESS MARKERS IN TYPE 2 DIABETES PATIENTS.

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Abstract:

Background &Method: The aim of the study is to find association between serum triglycerides and serum HNF1α with oxidative stress markers in type 2 diabetes patients. The microplate provided in this kit has been pre-coated with an antibody specific to HNF1α. study was conducted in the department of Biochemistry at the Sanotsh medical college & Hospital, Ghaziabad (U.P.). It is an observational case control study. Over 150 individuals from the Outpatient Department of Medicine at the Sanotsh Medical College in Ghaziabad participated in the current study. Out of 150 subjects selected, 75 were of T2DM, and 75 Normal healthy subjects which served as control group Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to HNF1α. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated.

Result: In our study we found 66.6% male & 33.4% female. In our study we found maximum no in age group of 51-60 (33.3%), followed by 41-50 (30.6%). Mean+SD in BMI kg/mt2 25.1725±5.34854. Mean+SD in HNF1A ng/ml 2.9943±1.89860. The chi-square statistic is 161.2834. The *p*-value is < 0.00001. The result is significant at $p < .05$.

Conclusion: This study shows that there is a significant correlation between diabetes mellitus and oxidative stress marker's. It is found that FBS, PPBS, HbA1c levels are significantly higher in diabetic patients as compared to control. Antioxidants (Catalase, SOD, GPX) were reduced significantly and oxidants (LPO, GR, GSH) were significantly higher in diabetic patients as compared to healthy controls. Glycated hemoglobin was positively correlated with high triglyceride. HbA1c can be an indicator of triglyceride level and can be one of the predictors of cardiovascular risk factors in type 2 DM. HNF1A mutations are associated with an increase in alpha cells in human pancreatic islets and differentiation of stem cell-derived beta cells toward alpha cells, showing the relevance of studies using human islets and beta cells.

Keywords: serum triglycerides, type 2DM, serum HNF1 α.

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Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by the elevated levels of glucose in the blood and insufficient secretion or action of endogenous insulin.[1] A report by the International Diabetes Federation in 2017 showed the worldwide prevalence of diabetes in the adult population reached 8.8% (424.9 million people).[2] Among them, the majority (87%-91%) of the cases are with type 2 diabetes. Globally, type 2 diabetes mellitus (T2DM) is considered a major public health concern because of its life-threatening complications with the increasing risk of mortality.[3] Although the exact etiologies of T2DM are not well defined, it is believed that autoimmune disease, genetic, and environmental factors play a major role in developing T2DM.[4] Also, recent studies have shown that with the high level of free-radical generation, oxidative stress (OS) initiates a significant role in developing and progressing T2DM.[5, 6, 7, 8]

OS can be defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense by which the body can detoxify its harmful effects and inhibit cell damages. The generation of ROS was thought to be a form of pathological cellular stress, but the current investigation is that ROS formed due to the physiological and homeostatic functions of many cells. However, an excess formation of ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals can cause harmful effects on many cellular structures such as protein, lipids, and nucleic acids.[9] Antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPX) counter the action of ROS by neutralizing their action or by inhibiting their formation.[10] Thus, a balance might be important between ROS and the levels of the antioxidant; otherwise, OS has been implicated in the pathogenesis of a variety of diseases, including cancer, obesity, diabetes mellitus, cardiovascular diseases, and nonalcoholic fatty liver disease.[11, 12] Various studies have reported that significant and abnormal increases in the levels of OS biomarkers associated with T2DM.[13, 14, 15, 16, 17, 18, 19, 20, 21]

HNF1α are transcription factors expressed in hepatocytes, renal tubular cells, intestinal epithelial cells, andpancreatic β-cells. Transcription factors are regulatory proteins that generally consist of a DNA-binding domain that specifically recognizes a short DNA sequence of about 10 base pairs. It also contains an activation or repression domain that affects gene transcription. The HNF1α protein essentially consists of three functional domains: a dimerization domain (N-terminus), a DNA-binding domain and a transactivation domain (C-terminus) (Yang *et al*., 2016).

The HNF1α is the most commonly mutated gene in diabetics, with over 75 different mutants identified in this gene. About 2/3 of HNF1α mutants are missense mutants, and most other mutants produce proteins that are truncated at the C-terminus by a point mutation resulting in a stop codon (nonsense mutation) or more commonly a deletion/insertion of frameshift-nucleotides that result in a mutational event downstream of the altered amino acid sequence. Of the 52 missense mutations identified in the HNF1 gene, 44 different amino acid positions are involved and 31 are at the fully conserved amino acid position, primarily in the conserved dimerization and DNA-binding domains. Mutations at non-conservative positions of the HNF1 protein can profoundly affect the structure and function of the protein.

Heterozygous mutations in the HNF4α gene not only decreased insulin secretion leading to diabetes in early adolescence and early adulthood, but also lead to increased insulin secretion during the fetal, neonatal, and infantile periods (Arya*et al*., 2014). The consequences of the increased insulin secretion range from fetal macrosomia to persistent hyperinsulinism.

Material & Method

Study population and design:

This study was conducted in the department of Biochemistry at the Sanotsh medical college & Hospital, Ghaziabad (U.P.). It is an observational case control study. Over 150 individuals from the Outpatient Department of Medicine at the Sanotsh Medical College in Ghaziabad participated in the current study. Out of 150 subjects selected, 75 were of T2DM, and 75 Normal

healthy subjects which served as control group. All subjects with T2DM were diagnosed by the criteria of American Diabetes Association. The study commenced after obtaining due approval from Institutional Ethical committee. Each and every participant received prior counseling regarding diabetes, causes, symptoms, complications etc. All the subjects were informed of the objectives of the study prior to registration. All subjects were given full disclosure of the study's benefits and drawbacks before providing their written consent.

❖ Inclusion criteria employed for selection of subjects

Type 2 diabetes subjects

- Males and females with type 2 diabetes mellitus in the age group of 35 to 55 years.

- Subjects having body mass index (BMI) between 18.5 and 40.
- Elevated blood glucose levels (fasting blood glucose ≥ 126 mg/dl and postprandial blood glucose ≥ 200 mg/dl). Participants in the study must be willing to follow the procedure and give written informed consent.

Healthy Volunteers
Male or female subjects aged 35 to 55 years with sound health (age matched to subjects with T2DM)

❖ **Exclusion criteria employed for selection of subjects**

Type 2 diabetes subjects
- Those with any kind of chronic illness or diseases or disorders
- Pregnant females and lactating women were excluded from the study.
- Those on steroid therapy for other ailments.
- Those addicted to alcohol or other drugs

Healthy Volunteers
Those with any kind of chronic illness or diseases or disorders &Those addicted to alcohol or other drugs

Estimation of Plasma glucose level was done by GOD-POD method; Trinder, 1969, Glycosylated hemoglobin by Ion Exchange Resin method; Trivelli *et al*., 1971, Estimation of triglycerides was done by GPO-PAP Method of Fossati **and** Prencipe, **1982**, Estimation of reduced glutathione was done by Ellman, 1959, estimation of Glutathione peroxidase assay done by Paglia and Valentine, 1967, Glutathione reductase by (Kamiya biomedical, KT-949), Superoxide dismutase (SOD) was done by Winterbourn*et al*., 1975, estimation of Catalase by (Sinha, 1972),Estimation of HNF-1α level was done by Raybiotech Inc., GA, USA.

Results:

Table No. 1: Demographic Distribution based on gender among diabetic & non-diabetic

S. No.	Sex	No.	Percentage (%)
1	Male	100	66.6
2	Female	50	33.4

Table No. 2: Demographic Distribution based on Age

S. No.	Age (Years)	No.	Percentage (%)
1	30-40	32	21.5
2	41-50	46	30.6
3	51-60	50	33.3
4	More than 60	22	14.6

Table No. 3: BMI Distribution among diabetic & non-diabetic

N=150	Mean	Std. Deviation	Minimum	Maximum
BMI kg/mt2	25.1725	5.34854	14.69	38.29

Table No. 4: HNF1A ng/ml

N=150	Mean	Std. Deviation	Minimum	Maximum
HNF1A ng/ml	2.9943	1.89860	0.33	6.79

Table No. 5: Mean of Parametersamong diabetic & non-diabetic

Parameters (N=150)	Mean	Std. Deviation	Minimum	Maximum
Catalase Unit/ ml	11.3990	3.48922	4.60	19.78
SOD unit/mg protein	3.7370	2.00855	.56	11.94
LPO (nmol MDA/ml)	5.2367	1.71965	1.08	9.66
GR (unit/min/mg protein)	1.0519	.34979	.32	2.29
GPX (nmol NADPH oxidized/min/mg protein	28.9850	4.98264	11.24	45.15
GSH umolGSH/mg protein	1.8060	.81097	.52	5.29
Fasting Blood sugar mg/dl	176.7913	66.55242	80.00	439.00
PP Blood Sugar mg/dl	258.9320	97.28087	94.00	580.00
HbA1C %	9.2470	2.86107	4.70	16.10
TG mg/dl	183.4591	109.57875	60.00	714.00
HNF1A ng/ml	2.9943	1.89860	0.33	6.79

Discussion:

In our study we found 66.6% male & 33.4% female were covered. In our study we found maximum no in age group of 51-60 (33.3%), followed by 41-50 (30.6%). Mean+SD in BMI kg/mt2 25.1725±5.34854. Mean+SD in HNF1A ng/ml 2.9943±1.89860. The chi-square statistic is 161.2834. The *p*-value is < 0.00001. The result is significant at $p < .05$. This study shows that there is a significant correlation between diabetes mellitus and oxidative stress marker's. It is found that FBS, PPBS, HbA1c levels are significantly higher in diabetic patients as compared to control. Antioxidants (Catalase, SOD, GPX) were reduced significantly and oxidants (LPO, GR,GSH) were significantly higher in diabetic patients as compared to healthy controls. Diabetes is a multifactorial disorder having a wide range of lipid abnormalities. In type 2 diabetes mellitus, there is an increased incidence of hypertriglyceridemia as compared to other lipid abnormalities [22-23]. This study evaluated the correlation between glycated hemoglobin (HbA1c) and triglycerides level and the results showed that there is a significant correlation between high HbA1c and high triglyceride. This may in turn help in predicting the triglyceride status of type 2 diabetics from the degree of glycemic control and therefore identifying patients at increased risk from cardiovascular events [24-25].

Conclusion:

There is a significant correlation between glycemic control and triglyceride levels in patients with type 2 diabetes in this population. Familiarity with this concept help to diagnose lipid abnormalities in patients with poor glycemic control and preventing cardiovascular events in the high-risk patient. Antioxidant levels could be taken as an early marker of the pathogenesis of complications in Type 2 Diabetes Mellitus. Therefore, supplementation of antioxidants may be a valuable strategy for controlling diabetes complications and enhancing antioxidant capacity. Findings indicate that HNF1A mutations are associated with an increase in alpha cells in human pancreatic islets and differentiation of stem cell-derived beta cells toward alpha cells, showing the relevance of studies using human islets and beta cells. The molecular basis of HNF1A abnormality in insulin secretion in human beta cells and the pathophysiological role of HNF1A in the liver, kidneys, and gut in diabetes requires further investigation. In this review, we discuss the similarities and differences in the pathophysiological role of HNF1A in patients with T2DM.

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