

## ORIGINAL ARTICLE



# Relationship between serum Transaminases with serum Neopterin in Type 2 Diabetes Mellitus with & without complication

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### Abstract

**Objective:** To know if serum neopterin level has any correlation with the Aspartate aminotransferase (AST) level & Alanine aminotransferase (ALT) level in patients with type 2 Diabetes Mellitus with and without complication (diabetic foot ulcer)

**Method:** An observational study conducted over a period of one year from December 2017 to December 2018 in 120 patients with type 2 diabetes mellitus categorized into two groups including newly detected type 2 Diabetes mellitus patients and those with diabetic foot ulcer. The blood samples of the patients were analysed in Clinical Biochemistry laboratory and the data obtained after estimation of analytes were statistically analysed.

**Result:** In the newly detected diabetes mellitus group, there was a significant positive correlation between the levels of neopterin and AST. In the diabetic foot ulcer group, there was a significant positive correlation between the levels of neopterin and ALT.

**Conclusion:** This study could establish a significant positive correlation between serum neopterin and AST in newly detected diabetic patients indicating its potential in using it as a biomarker in detecting cardiac events in newly detected diabetic patients. In diabetic foot ulcer group the significant correlation between Neopterin and ALT, could be used as an early indicator of Non-alcoholic fatty liver disease (NAFLD), a common complication in chronic Diabetes Mellitus.

**Keywords:** Neopterin, AST, ALT, type 2 diabetes mellitus, diabetic foot ulcers

## 1 | INTRODUCTION

In Diabetes mellitus, the body's ability to secrete or respond to the hormone insulin is impaired, this results in abnormal metabolism of carbohydrates and there is elevated levels of glucose in

the blood and urine. Prevalence of Type 2 diabetes mellitus is on the drastic rise.<sup>[1]</sup> Longevity in life expectancy in humans now contribute to the various complications of diabetes. Coronary artery disease (CAD) is a life threatening macrovascular complication of diabetes mellitus. Diabetic neuropathy

is a chronic microvascular complication of diabetes mellitus, as a consequence of this, the incidence of diabetic foot ulcers are on the rise.<sup>[2]</sup> Diabetic foot causes financial and psychological burden on the patients.

Neopterin is produced by activated monocytes, macrophages, and dendritic cells when stimulated by interferon gamma which is synthesized by T-lymphocytes (Figure 1). Neopterin level increases as a result of Systemic immune activation. Neopterin levels increase proportionally with the progression of the disease from prediabetes to type 2 diabetes.<sup>[3]</sup> So Neopterin is a useful inflammatory marker of diabetes progression.<sup>[4]</sup>

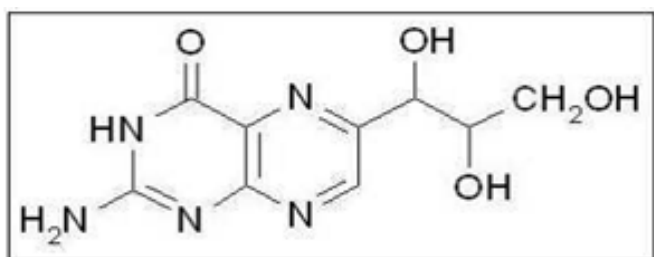


FIGURE 1: Structure of Neopterin

The two main metabolites in the tetrahydrobiopterin (BH<sub>4</sub>) pathway are neopterin and biopterin. BH<sub>4</sub> is synthesized de novo from GTP through the actions of GTP HYPERLINK "https://www.sciencedirect.com/topics/medicine-and-dentistry/guanosine-triphosphate-cyclohydrolase-i" cyclohydrolase I (Figure 2). Inflammatory cytokines such as interferon-gamma induces the activity of the enzyme GTP cyclohydrolase I and so macrophages are one of the main producers of neopterin.<sup>[5]</sup>

The possibility of using Neopterin as a biomarker in CAD was identified in 2015.<sup>[6]</sup> and as a marker for Non alcoholic fatty liver disease (NAFLD) was

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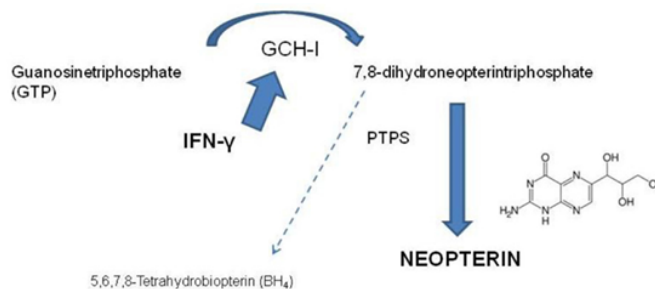


FIGURE 2: Synthetic pathway of Neopterin

identified in 2005. Neopterin as an inflammatory marker can be used in predicting diabetic complications. Based on this we aim at comparing the serum neopterin levels with the level of AST & ALT in two groups namely newly detected type 2 Diabetics with no complications and those with diabetic foot ulcer an established complication.

## 2 | MATERIALS & METHOD

Study setting:

- Sree Gokulam Medical College and Research Foundation
- Department of Biochemistry, Surgery and Diabetology

Study design:

Observational study

Study period:

December 2017-November 2018

Sample size:

120 patients; 60 with type 2 Diabetes mellitus (detected within last 6 months) and 60 patients with diabetic foot ulcer.

Study population:

Diabetes mellitus patients attending the Diabetology out patient department and diabetic foot ulcer patients from the surgery ward

Ethical consideration:

The study was carried out after approval of institutional research & ethical committee of Sree Gokulam Medical College & Research Foundation. This study is done after obtaining informed written consent from the study subjects.

Selection criteria:

Inclusion criteria:

- Patients with Type 2 DM newly detected as per ADA guidelines
- Patients with diabetic foot ulcers

Exclusion criteria:

Patients with,

- Coronary artery disease(CAD)
- Malignancy
- Any other infectious diseases at present
- Obese patients
- Chronic liver disease(CLD)

Study variables:

- Neopterin level
- AST level
- ALT level

Specimen collection:

3 ml of blood was collected from patients attending the Diabetology OPD and surgery ward after getting the informed consent

Specimen storage:

Samples were stored at -20 degree Celsius in deep freezer

Assays:

Neopterin Assay:

- Principle:

Human Neopterin Elisa (enzyme-linked immune sorbent assay (ELISA) kit uses Biotin labelled double antibody sandwich technology and can estimate human NEOP levels in various human sample types. Samples will be added to wells that are pre-coated with NEOP antibody. After incubation, anti-NEOP antibody labelled with biotin will be added along with streptavidin-HRP resulting in formation of immune complex. Unbound enzyme will be removed by washing. After incubation, substrate A and B will be added. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of human NEOP are positively correlated. The readings were taken from Bio-Rad Elisa Reader.

Reference range:

There is no difference between neopterin values detected in serum or plasma. On average the concentrations are 5.2±2.5 nmol/l neopterin. In our study the neopterin results are skewed as the lowest and the highest value are in the extreme ends, so mean cannot be taken. Median confidence interval is taken with the median being 1.26. So reference range in our population is 1.13-1.58 nmol/l according to the age of the participants in our study.

Serum AST Assay:

**Analyser:** Siemens AST flex reagent cartridge in SIEMENS dimension RxL max Integrated Chemistry System refurbished march 2012

**Principle:** Aspartate aminotransferase (AST) catalyzes the transamination from L-aspartate to alpha ketoglutarate, forming L-glutamate and oxaloacetate. The oxaloacetate formed is reduced to malate by malate dehydrogenase (MDH) with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the AST activity and is measured using a bichromatic (340, 700nm) rate technique. sample size-40 µl

Serum ALT assay:

**Analyser:** Siemens ALT flex reagent cartridge in SIEMENS dimension RxL max Integrated Chemistry System refurbished march 2012

**Principle:** Alanine aminotransferase (ALT) catalyzes the transamination of L-alanine to alpha ketoglutarate, forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD is directly proportional to the ALT activity and is measured using a bichromatic (340, 700nm) rate technique. sample size-35  $\mu$ l

### 3 | RESULTS & DISCUSSION

The data obtained after estimation of analytes were statistically analyzed with Microsoft excel and SPSS software version 18. A probability value of less than 0.05 is considered as the threshold for statistical significance. Quantitative variables are expressed as mean plus or minus standard deviation (SD). Correlation between two quantitative variables were studied by Pearson's correlation

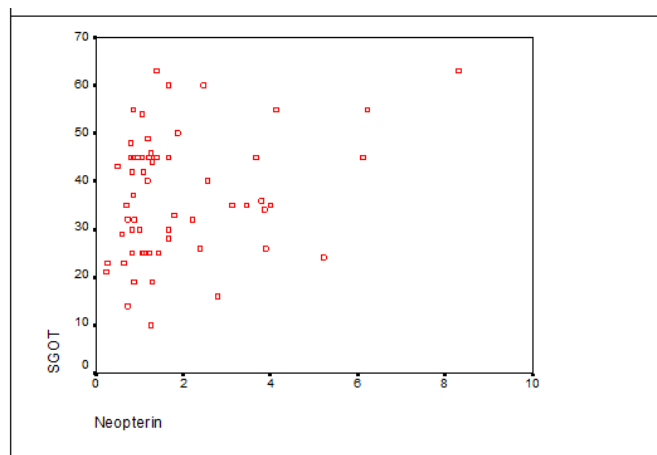
Neopterin and AST:

**TABLE 1: Correlation between AST and neopterin for both the groups by Pearson's correlation**

	Diabetic foot ulcer		Newly diagnosed diabetics	
	r	r	r	r
AST(SGOT)	0.192	0.142	0.273*	0.035

Significant at 0.05 level

Aspartate transaminase (AST) or serum glutamic oxaloacetic transaminase (SGOT), is a pyridoxal phosphate (PLP)-dependent transaminase enzyme. AST participates in the transamination reaction where it catalyzes the reversible transfer of an  $\alpha$ -amino group between aspartate and glutamate. AST is found in the liver cells, cardiac muscle, skeletal muscle, kidneys, brain, and red blood cells. Elevation of AST is seen in diseases affecting these organs, like myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma.



**FIGURE 3: Scatter diagram for AST(SGOT) and neopterin for newly diagnosed diabetics**

The value of neopterin was compared with AST level in both the group. Reference range was 15-37 U/L. In the diabetic foot ulcer group, the correlation between the level of AST and neopterin value was not statistically significant. In the newly detected diabetic group the level of AST and neopterin positively correlated and with the a p value of 0.035, the correlation was statistically significant. (Table II)

In a study by Muhammed Aydin et al. the relation between aspartate aminotransferase (AST) level and neopterin level was analyzed, in patients with viral hepatitis and a significant relation was detected between neopterin levels and AST ( $p < 0.001$ ). [7] In another study by Enver Mahir Gulcan et al. in the same group of patients significant correlations were found between serum neopterin levels and AST ( $P = 0.22$ ) level. [8]

In this present study a significant correlation was seen in the newly detected diabetic patients. AST and Neopterin are used as cardiac marker as well. The moderately increased serum neopterin concentrations in patients with diabetes mellitus probably reflect systemic immune activation associated with atherosclerosis. Atherosclerosis has been shown to be associated with increased neopterin levels in numerous studies. [9]

In the study of Chelapram Kandy Firoz et al. Neopterin as an immune biomarker of coronary artery disease and its association with other CAD markers, Neopterin was markedly raised in CAD patients. [6] In newly detected diabetics, the chronic in-

flammation phase starts setting in,so the correlation between AST and neopterin can be used as an early marker for the risk of developing coronary heart disease in newly diagnosed patients.

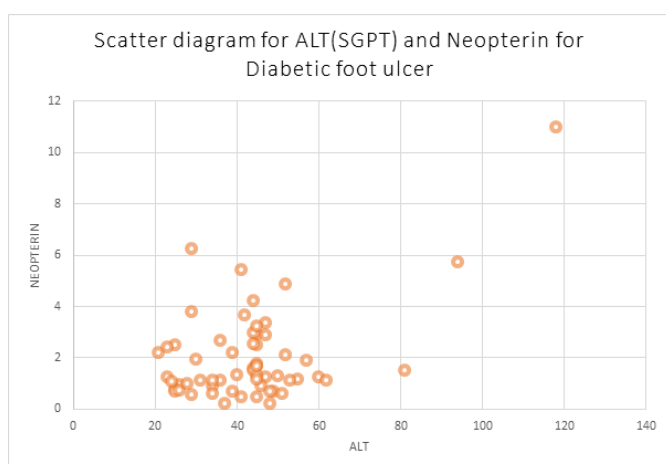
Neopterin and ALT:

**TABLE 2: Newly Diabetics :correlation between ALT & Neopterin by Pearson's correlation**

	SGPT	Neopterin
Pearson Correlation	1	.137
SGPT Sig. (2-tailed)		.295
	60	60
Pearson Correlation	.137	1
Ne Sig. (2-tailed)	.295	
	60	60

**TABLE 3: Diabetic foot ulcer:Correlation between ALT & Neopterin by Pearson's correlation**

	SGPT	Neopterin
Pearson Correlation	1	.509**
SGPT Sig. (2-tailed)		.0001
	61	61
Pearson Correlation	.509**	1
Ne Sig. (2-tailed)	.0001	
	61	61
** . Correlation is significant at the 0.01 level (2-tailed).		



**FIGURE 4:**

Alanine amino transaminase (ALT) or serum glutamate pyruvate transaminase (SGPT), is a pyridoxal phosphate (PLP)-dependent transaminase en-

zyme. ALT participates in the transamination reaction where it catalyzes the reversible transfer of an  $\alpha$ -amino group between alanine and glutamate. ALT is found in organ tissues especially liver and also found in kidney, skeletal muscle, myocardium, pancreas, spleen, and lungs. ALT is the most widely used clinical biomarker of hepatic disease as it is elevated in serum due to hepatic cellular necrosis and is used as a measure of liver function.

The value of neopterin was compared with ALT level in both the groups. Reference range was 19-33 U/L. In the diabetic foot ulcer group, the correlation between the level of ALT and neopterin value was statistically significant (Table IV). In the newly detected diabetic group the level of ALT and neopterin was not statistically significant (Table III)

One of the main cause of macrovascular steatosis in Non-alcoholic fatty liver disease (NAFLD) is Type 2 Diabetes Mellitus.<sup>[10]</sup> In a study by Nur Aslan et al Evaluation of serum neopterin levels and its relationship with adipokines in pediatric obesity-related non-alcoholic fatty liver disease and healthy adolescents.<sup>[11]</sup>, serum neopterin levels compared to controls were significantly higher in obese adolescents with fatty liver disease. But in the same study there was no correlation between neopterin level and ALT levels.

In a study by Halil Yaman et al Elevated urine neopterin levels in non-alcoholic steatohepatitis.<sup>[12]</sup>, the urinary neopterin levels were found elevated in the Non alcoholic Steatohepatitis group (NASH) group compared to the controls. Non-alcoholic fatty liver disease (NAFLD) is associated with the inflammation of liver cells and neopterin is regarded as a biochemical marker of cell-mediated immunity, So a well known marker of liver inflammation like ALT can be compared to that of Neopterin in both groups.

#### 4 | CONCLUSION:

- AST and Neopterin level shows significant positive correlation in the newly detected diabetic group but did not show any correlation in the diabetic foot ulcer group

- ALT and Neopterin level shows significant correlation in the Diabetes with complication group (Diabetic foot ulcers) but did not show any correlation in the newly detected diabetes group

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