(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 6, Issue 2 , Page No: 306-313
March-April 2023

IJMSCR

Serum Neopterin As An Inflammatory Marker In Diabetic Foot Ulcer

[1]Kezia Blessinda. R, [2]Meetha. S.S, [3]Saleena Prameela.C.R ,[4]Sreekumari. S
[1&2]Assistant Professor ,[3&4]Professor
Sree Gokulam Medical College & Research Foundation

***Corresponding Author:**
Kezia Blessinda. R
Assistant Professor, Sree Gokulam Medical College & Research Foundation

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Background:

This is 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 objective of the study was to find out if serum neopterin level is elevated in patients with diabetic foot ulcers compared to newly detected diabetics and the clinical usefulness of Neopterin as a biomarker compared to an existing inflammatory marker like CRP.

Method:

The blood samples of the patients were analyzed in Clinical Biochemistry laboratory and the data obtained after estimation of analytes were statistically analyzed.There is no statistically significant difference in the neopterin level in both groups.(P value is 0.625).Comparison between neopterin and CRP in both the groups showed a statistically significant negative correlation (p value is 0.048).

Conclusion:

This study could not establish a significant correlation between serum neopterin level in newly detected diabetics & diabetic foot ulcer patients indicating that it cannot be used as a biomarker.

Keywords: Neopterin,type 2 diabetes mellitus,diabetic foot ulcers

Introduction

Diabetes mellitus is undoubtedly one of the most important public health challenge we face globally, as its prevalence has more than doubled over the past 30 years. [1] India tops the list for countries estimated to have the highest numbers of people with diabetes followed by China and USA.[2]

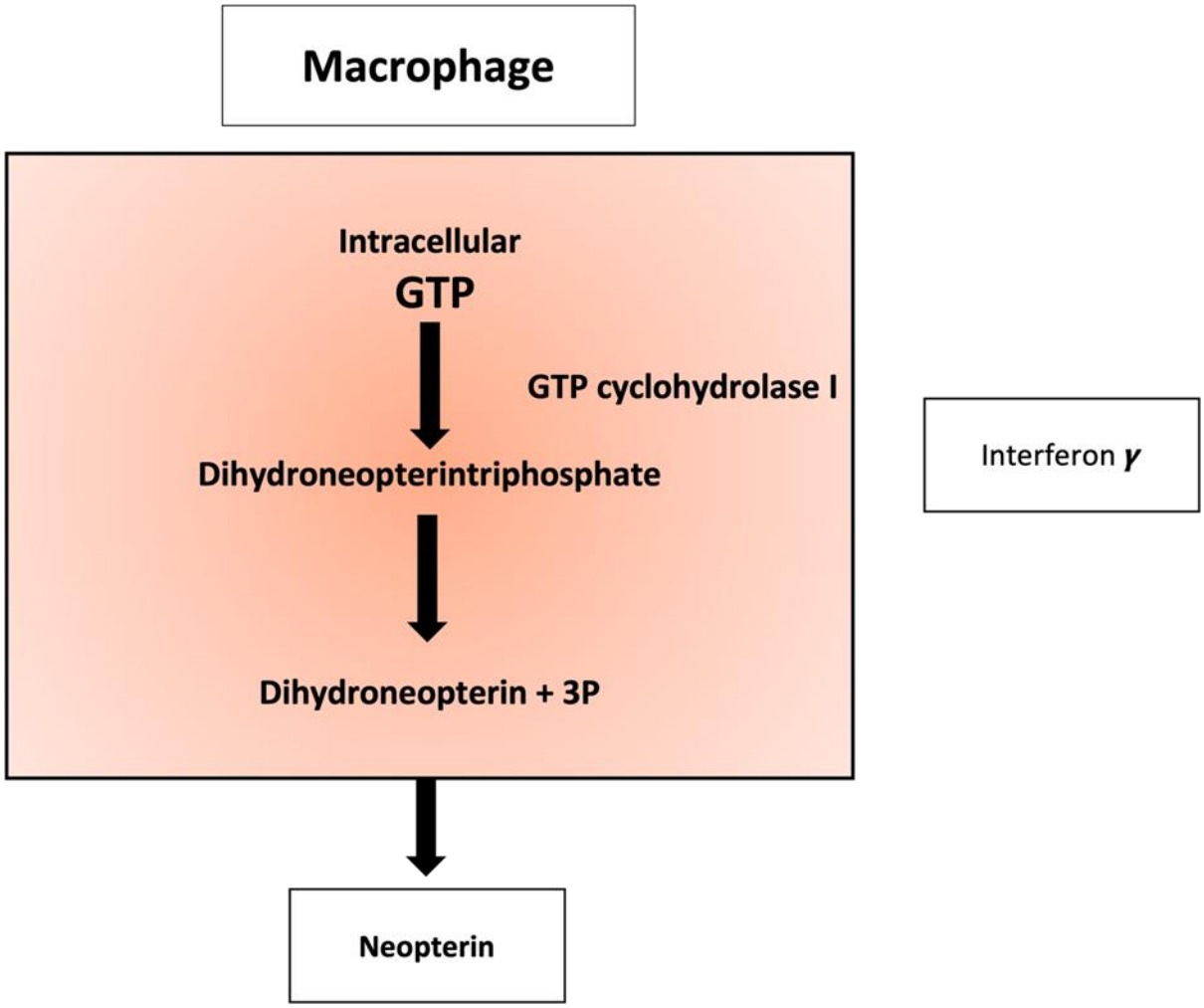
Diabetic foot ulcer is one of the most common predicament encountered during diabetic patient care. Diabetic foot ulceration is defined as the full-thickness penetration of the dermis of the foot in a person with diabetes. It's severity is graded from 1 to 5 according to Wagner system. In resource rich nations the incidence of ulcers in diabetic patients annually is 2.5% to 10.7% and the incidence of amputation due to any reason is 0.25% to 1.8% annually. [3] Diabetic foot disease has a multifactorial etiology which comprises poor glycemic control along with complications of diabetic neuropathy, vasculopathy, and immunopathy. The most common cause of diabetic foot ulcers is diabetic neuropathy which leads to sensory, motor and autonomic dysfunction. [4] A common cause of morbidity in the diabetic patient, diabetic foot ulcers impose significant burden psychologically and financially to the patient as well as the society.

Neopterin is a compound derived from GTP by the action of GTP cyclohydrolase I. Interferon gamma

increases the synthesis of neopterin by inducing the activity of cyclohydrolase I.[5]

Macrophages and monocytes in human beings lack the enzyme 6-pyruvoyltetrahydropterin synthetase required for the conversion of NH2TP to 6-pyruvoyltetrahydropterin. Hence NH2TP accumulate in these cells which is further acted upon by phosphatases and is excreted as neopterin or dihydroneopterin. Neopterin production was found to be associated with activation of the cellular immune system. Elevated levels of neopterin was seen in some malignancies and various inflammatory diseases. It is a marker that can be easily assayed in serum as well as urine. [6] Hence to evaluate the role of neopterin as an inflammatory marker for predicting diabetic complication, in this study we aim at comparing the serum neopterin levels in newly detected type 2 diabetes mellitus patients with no complications and those with diabetic foot ulcer as an established complication & compare it with a known inflammatory marker like the CRP.

Figure 1:Synthesis of Neopterin



CRP is one of the several biomarkers that are elevated in type 2 Diabetes mellitus owing to the low grade inflammation that is seen in the condition.High glucose,adipokines,modified lipoproteins and free fatty acids are some of the inflammatory and metabolic factors associated with diabetes.These factors triggers the endothelial cells,smooth muscle cells and monocytes/macrophages to produce CRP.Newer studies done shows that CRP along with ESR is more helpful than CBC in diabetic foot ulcer

Materials & Methods:

Volume 6, Issue 2; March-April 2023; Page No 306-313

Study Setting:

1. Sree Gokulam Medical College and Research Foundation
2. 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:

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

Exclusion Criteria:

Patients with,

1. Coronary artery disease(CAD)
2. Malignancy
3. Any other infectious diseases at present
4. Obese patients
5. Chronic liver disease(CLD)

Study Variables:

1. Neopterin level
2. CRP level

Specimen Collection:

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

Specimen Storage:

Samples were stored at -20 degree Celsius in deep freezer

Assays:

Neopterin Assay:

Principle:

Human Neopterin ELISA (enzyme-linked immunosorbent assay)kit uses Biotin labelled double antibody sandwich technology and can estimate human neopterin levels in various human sample types.

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 were skewed as the lowest and the highest value are in the extreme ends,so mean could not be taken,instead median confidence interval was 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 Crp:

Analyzer:Bayer's Immunoturbidometry

Principle:TURBILYTE-CRP is a turbidimetric immunoassay for the determination of C-reactive protein in human serum and is based on the principle of agglutination reaction.

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

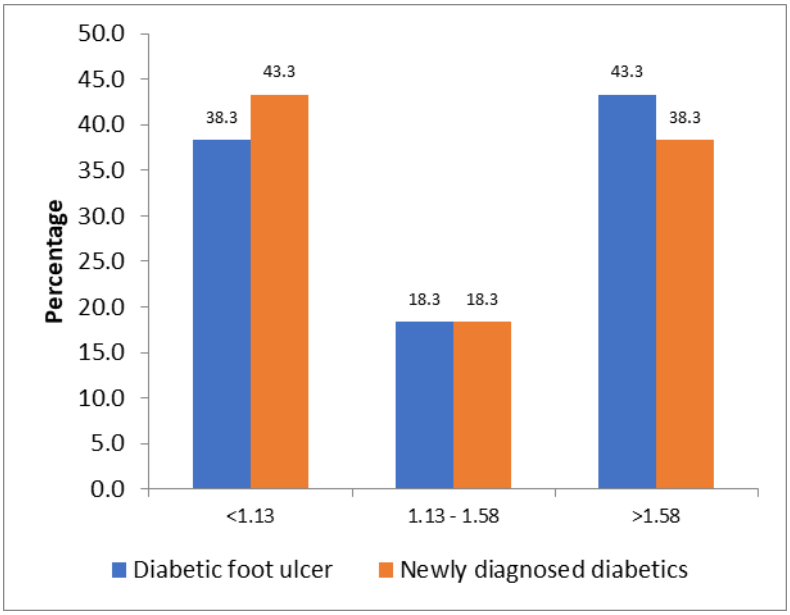
1.Comparison of Serum neopterin level in patients with Diabetic foot ulcers and newly detected Diabetes mellitus.

Volume 6, Issue 2; March-April 2023; Page No 306-313

Table 1 Distribution of neopterin based on group,

Neopterin	Diabetic foot ulcer		Newly diagnosed diabetics	
	Frequency	Percent	Frequency	Percent
<1.13	23	38.3	26	43.3
1.13 - 1.58	11	18.3	11	18.3
>1.58	26	43.3	23	38.3

Fig 2. Distribution of neopterin based on group



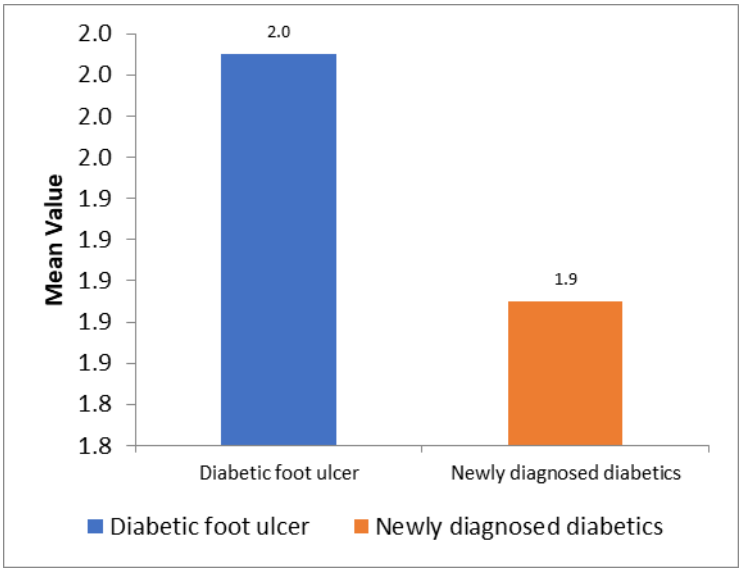
Analysis:The reference range of neopterin in our study is 1.13-1.58 nmol/ml.On comparing both the groups,the neopterin concentration of less than 1.13nmol/ml is seen more in newly detected diabetic patients.The normal range is seen equally in both the groups.Elevation of neopterin more than 1.58nmol/ml is seen more in patients with diabetic foot ulcer.

Table Comparison of neopterin based on group,Table:2

Group	Mean	SD	N	Median (IQ Range)	Z#	p
Diabetic foot ulcer	2	1.8	60	1.3 (0.92 - 2.53)	0.49	0.625
Newly diagnosed diabetics	1.9	1.6	60	1.2 (0.85 - 2.43)		

Volume 6, Issue 2; March-April 2023; Page No 306-313

Fig. 3 Comparison of neopterin based on group



Analysis:There is no significant difference in the neopterin level in both groups.

2.Correlation between Neopterin and CRP in patients with diabetic foot ulcer when compared to newly diagnosed diabetes

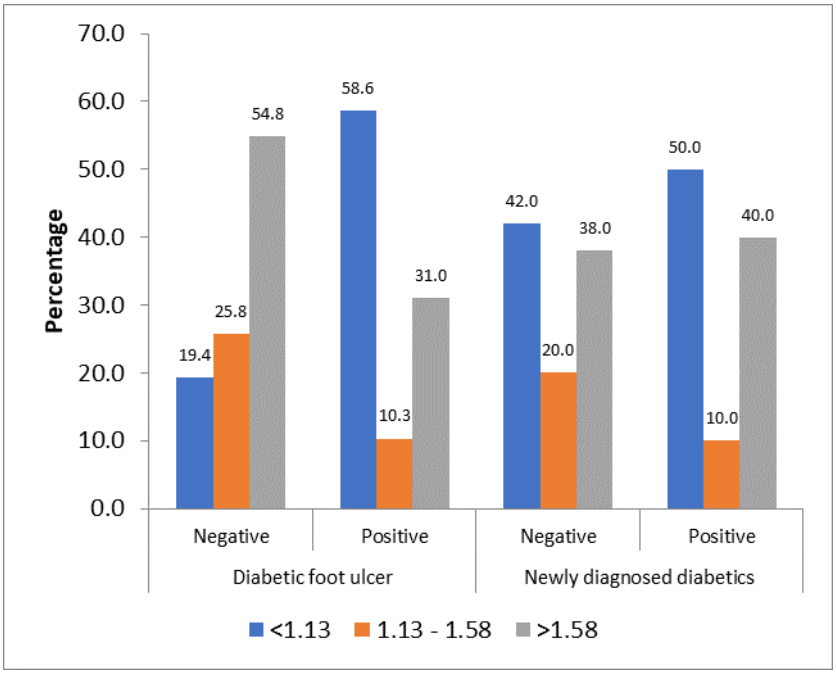
Table Comparison of CRP based on neopterin for different group by Chi-square test;Table:3

Group	CRP	<1.13		1.13 - 1.58		>1.58		χ^2	p
		frequency	Percent	frequency	Percent	frequency	Percent		
Diabetic foot ulcer	Negative	6	19.4	8	25.8	17	54.8	9.94*	0.007
	Positive	17	58.6	3	10.3	9	31		
Newly diagnosed diabetics	Negative	21	42	10	20	19	38	0.59	0.746
	Positive	5	50	1	10	4	40		

**: - Significant at 0.01 level

Volume 6, Issue 2; March-April 2023; Page No 306-313

Figure:4 : Comparison of CRP based on neopterin for different group



Analysis:To know the efficiency of Neopterin as a biomarker ,we compared its value on the study subjects with a known biomarker C-Reactive protein.A CRP value of more than or equal to 0.6 was taken as positive and less than 0.6 as negative.

Table Correlation between neopterin and CRP in patients with diabetic foot ulcer when compared to the newly diagnosed diabetics done by Pearson's correlation test.Table:4

Group	r	p
Diabetic foot ulcer	-0.256	0.048*
Newly diagnosed diabetics	-0.027	0.841

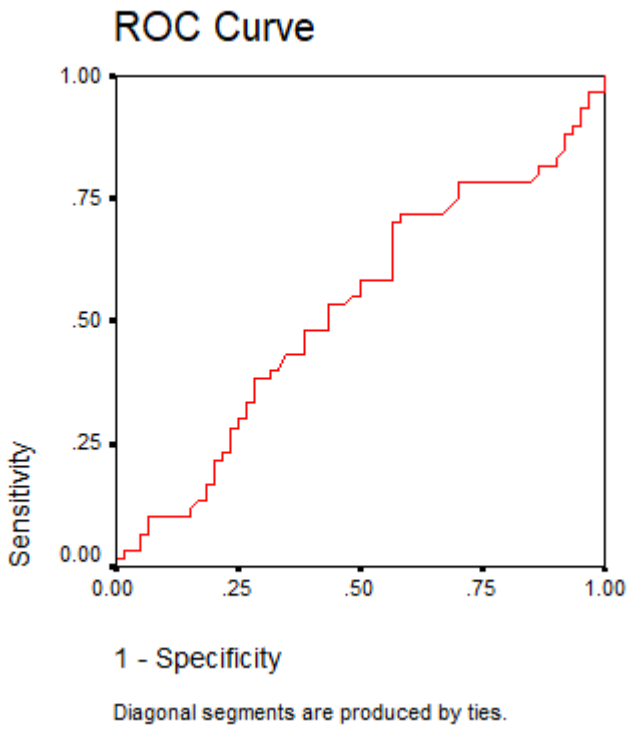
*: - Significant at 0.05 level

Analysis:The comparison between neopterin and CRP in both the groups was studied by Pearson's correlation and it showed a statistically significant negative correlation in diabetic foot ulcer group.

ROC CURVE

Volume 6, Issue 2; March-April 2023; Page No 306-313

ROC curve in predicting diabetic foot ulcer using neopterin Fig.5



Area under curve with 95 % CI – 0.526 (0.421- 0.630), p=0.625

Area under the curve=0.526

For good prediction the area under the curve should be more than 0.8

This shows the predictive power of neopterin is not statistically significant.

Discussion:

CRP is an established biomarker for systemic inflammation.As Neopterin is a fairly new biomarker,the sensitivity of neopterin is compared with a known biomarker like CRP.In both the groups the level of CRP and neopterin were compared.A CRP value of more than or equal to 0.6 was taken as positive and less than 0.6 as negative.

According to Pearson's correlation in the second group with diabetic foot ulcer CRP and neopterin showed negative correlation with a p value of 0.048,indicating a statistically significant correlation.That is as the CRP value increased the neopterin value decreased and vise versa.

In a study by Alicia Lacoma,CristinaPrat et al. [7]The levels of CRP and neopterin as biomarkers were compared in 3 groups,COPD patients who were in the stable phase,undergoing an exacerbation, and those with pneumonia.CRP levels showed significant differences among the 3 groups of patients, being lower during clinical stability and higher during pneumonia (*P*< 0.0001)and in contrast, neopterin levels did not show any significant difference.In the same study,CRP levels decreased 1 month after the exacerbation episode of COPD, while neopterin increased. Neopterin showed significantly lower levels in exacerbations

Manna et al.[8] investigated children and young adults with diabetes and found increased urinary neopterin in patients with newly diagnosed diabetes, while normal concentrations were observed in subjects more than 1 year after diagnosis.In this study the level of neopterin didn't show significant correlation in foot ulcer patients,In our parent study[9] also there was no significant elevation of neopterin in ulcer patients.Hence with the above evidence & our study result it is safe to say that Neopterin cannot be used as an inflammatory marker.

Volume 6, Issue 2; March-April 2023; Page No 306-313

PREDICTIVE POWER OF SERUM NEOPTERIN ASSAY:

In the present study the predictive power of serum neopterin assay as a marker for diabetic foot ulcer was studied.Accordingly a receiver operating charateristics(ROC)curve was constructed based on the serum neopterin concentration.The area under the ROC curve of serum neopterin was 0.526,for a good prediction the area should be atleast more than 0.8.With a p value of 0.625 ,the predictive power of serum neopterin in diabetic foot ulcer is not statistically significant.So it can be said that serum neopterin cannot be used as a marker for early diagnosis or prediction of diabetic foot ulcers.

Conclusion:

1. Among biochemical variables,serum neopterin did not show any significant correlation between the two groups-newly detected diabetics and diabetic foot ulcer patients.
2. CRP and Neopterin did not have significant correlation in the newly detected diabetic group but in the diabetic foot ulcer group,the level of CRP and neopterin showed negative correlation.

Limitations:

1. All subjects in this study were diabetic patients,normal control were not included in this study
2. Ulcer patients were not separated with peripheral artery disease or without peripheral vascular disease
3. No evidence suggesting super added bacterial infection in diabetic foot ulcers were done

Reference

1. Chen, L., Magliano, D. & Zimmet, P. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. *Nat Rev Endocrinol* **8**, 228–236 (2012) https://doi.org/10.1038/nrendo.2011.183
2. Sarah Wild, Gojka Roglic, Anders Green, Richard Sicree, Hilary King; Global Prevalence of Diabetes: **Estimates for the year 2000 and projections for 2030**. *Diabetes Care* 1 May 2004; 27 (5): 1047–1053. https://doi.org/10.2337/diacare.27.5.1047
3. Hunt, Dereck L. "Diabetes: foot ulcers and amputations." *BMJ clinical evidence* vol. 2011 0602. 26 Aug. 2011
4. Del Core MA, Ahn J, Lewis RB, Raspovic KM, Lalli TAJ, Wukich DK (2018) The evaluation and treatment of diabetic foot ulcers and diabetic foot infections. Foot Ankle Orthopaed 3(3):247301141878886
5. Schoedon G, Troppmair J, Adolf G, Huber C, Niederwieser A. Interferon-gamma enhances biosynthesis of pterins in peripheral blood mononuclear cells by induction of GTP-cyclohydrolase I activity. J Interferon Res. 1986 Dec;6(6):697-703. doi: 10.1089/jir.1986.6.697. PMID: 3106526.
6. Hamerlinck, F.F.V. (1999), Neopterin: a review. Experimental Dermatology, 8: 167-176. https://doi.org/10.1111/j.1600-0625.1999.tb00367.x
7. Alicia Lacoma,CristinaPrat, Felipe Andreo, Luis Lores,Juan Ruiz-Manzano, Vicente Ausina,and Jose Domínguez "Value of procalcitonin, C-reactive protein, and neopterin in exacerbations of chronic obstructive pulmonary disease"Int J Chron Obstruct Pulmon Dis. 2011; 6: 157–169
8. Manna R, Gambassi G, Papa G, et al. Urinary neopterin levels of insulin dependent diabetes (IDDM) at onset: In: Pfleiderer W, Wachter H, Blair JA, eds. Biochemical and Clinical Aspects of Pteridines. Walter de Gruyter: Berlin, 1987: 353 -357.
9. Karolina Melicharova, Marie Kusalova, Alena Smahelova, RadomirHyspler, Dagmar Solichova,BohuslavMelichar.Urinary Neopterin in Patients with Diabetes Mellitus and Foot Ulcers. Pteridines Vol. 18, 2007, pp. 128 – 131.

Volume 6, Issue 2; March-April 2023; Page No 306-313