



Original Article

Type 2 diabetes mellitus with early phase acute inflammatory protein on serum protein electrophoresis

Tuladhar ET¹, Sharma VK¹, Sigdel M², Shrestha L¹

¹Department of Biochemistry, Tribhuvan University Teaching Hospital, Kathmandu, Nepal

²Department of Biochemistry Department, Manipal College of medical Sciences, Pokhara, Nepal

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ABSTRACT

Background: The onset of Type 2 diabetes has been associated with low grade systemic inflammation. The inflammatory status has been studied by measuring acute phase reactant proteins like hsCRP, α 1-antitrypsin, α 1-acid glycoprotein, ceruloplasmin, fibrinogen. Most of these acute phase reactants form α 1 and α 2 bands on electropherogram of serum proteins. The aim of this study was to evaluate inflammatory status in controlled and uncontrolled type 2 diabetes using cellulose acetate electrophoresis and to find the impact of glycemic status as indicated by HbA1c on inflammation process.

Materials and Methods: Serum protein electrophoresis was done on serum samples of 60 cases of Diabetes [controlled and uncontrolled] using cellulose acetate paper technique. The electropherogram obtained was stained with Ponsu S and then quantitated using densitometer. Glycemic status was studied by HbA1c analysis. The density of α 1 and α 2 bands in electropherogram were correlated with HbA1c level.

Result: A significant increase in the percentage of α 1 and α 2 band proteins (0.765 and 0.716, $p < 0.001$) were found with the increasing level of HbA1c. With cutoff of HbA1c 7% (American Diabetic Association recommended), the α 1 and α 2 serum proteins concentration are significantly higher ($p < 0.001$) in uncontrolled diabetes mellitus compared to controlled diabetes mellitus.

Conclusion: Cellulose acetate electrophoresis of serum proteins show early phase acute inflammatory status in uncontrolled type 2 diabetes mellitus. The process of systemic inflammation worsens with uncontrolled glycemia as indicated by HbA1c. Inflammatory status should be studied adjunct to glycemic status.

INTRODUCTION

According to International Diabetic Federation (IDF) 2011, diabetes mellitus (DM) is a global pandemic affecting 8.3%

of the world's adult population. Prevalence of DM in Nepal is estimated as 3% in 2011.¹ Chronic hyperglycemia leads to microvascular complications like retinopathy, nephropathy, neuropathy as well as macrovascular complications like atherosclerosis.² Atherosclerosis leads to morbidity states like angina, myocardial infarction, stroke and peripheral vascular disease.² Type 2 DM is more prevalent having adult onset and characterized as insulin resistance which might be shared with low insulin secretion. Development

Correspondence:

Dr. Eans Tara Tuladhar, MD

Department of Biochemistry, Tribhuvan University Teaching Hospital, Kathmandu, Nepal

Post box: 1524

Email: eanstara@gmail.com

Table1: Serum protein bands of electropherogram in controlled and uncontrolled Diabetes Mellitus

	HbA1c	Number	Mean%	SD	SEM	95% confidence interval for Mean
Albumin	≤ 7.0	30	54.8	5.6	1.0	52.7 - 56.9
	>7.0	30	53.5	5.8	1.0	51.3 - 55.7
	Total	60	54.2	5.7	0.7	52.7 - 55.6
α1-band	≤ 7.0	30	3.54	0.4	0.1	3.4 - 3.7
	>7.0	30	6.3	3.8	0.7	4.8 - 7.7
	Total	60	4.9	3.0	0.4	4.1 - 5.7
α2-band	≤ 7.0	30	7.6	1.8	0.3	6.9 - 8.3
	> 7.0	30	10.5	4.3	0.8	8.9 - 12.1
	Total	60	9.0	3.6	0.4	8.1 - 10
β-band	≤ 7.0	30	8.8	1.8	0.3	8.1 - 9.5
	> 7.0	30	9.9	5.9	1.1	7.7 - 12.1
	Total	60	9.3	4.4	0.5	8.2 - 10.5
γ-band	≤ 7.0	30	25.3	6.5	1.2	22.8 - 27.7
	> 7.0	30	19.7	8.6	1.5	16.5 - 22.9
	Total	60	22.5	8.0	1.0	20.4 - 24.6

of Type 2 DM has been associated with low grade systemic inflammation. This low grade systemic inflammation releases Tumor Necrosis Factor (TNF); which make cells more resistant to insulin leading to diabetes. This inflammation is associated with hyperglycemia and glycated end products.³⁻⁶ The outcome of inflammation is microvascular and macrovascular complications like in retina, kidney, nerves and heart. Inflammatory markers like hs-CRP, α1-antitrypsin, α1- acid glycoprotein, ceruloplasmin, C4, C3 and C-reactive protein have been independently studied proving the association.⁷ These inflammatory markers are called acute phase proteins (APP). Others (such as transthyretin, albumin and transferrin) are decreased and known as negative acute-phase proteins.

In general, concentration of these plasma protein changes are helpful in detecting inflammation and can often be used to monitor the progress of the inflammation or its response to treatment. The increment and decrease of these APPs have been depicted in traditional serum protein electrophoresis. Generally five bands are shown in the cellulose acetate sheet electrophoresis of serum proteins. They are Albumin, α1, α2, β- and γ-bands starting from anode to cathode ends due to variability in the mobility of different serum proteins and quantification by densitometer. Acute phase reaction is associated with increase in α1- and α2- bands suggesting increase in positive APPs and thus indicating systemic inflammation.⁸⁻¹⁰

The aim of this study was to evaluate inflammatory status in controlled and uncontrolled type 2 DM using cellulose acetate electrophoresis and to find the impact of glycemic status as indicated by HbA1c on inflammation process.

MATERIALS AND METHODS

This was a prospective study conducted over three months duration from July 2011 to September 2011 in diabetic patients (controlled and uncontrolled) attending to

Tribhuban University Teaching Hospital.

The patients were chosen irrespective of their mode of treatment. Informed consent was taken from the patients. To rule out the confounding effects of preexisting atherosclerosis or renal impairment in causing an elevation of APPs, diabetic patients with clinical evidence of diabetic complications were excluded from the study. There were a total of 60 known diabetic patients (controlled, n=30 and uncontrolled, n=30), taking HbA1c = 7% as cut off point, as per ADA recommendation.

Serum protein electrophoresis was carried out on cellulose acetate sheet with quantification by densitometer as per methodology mentioned in Practical Clinical Biochemistry by Varley.¹³

Serum was separated from the collected blood sample. Serum protein electrophoresis was then conducted on cellulose acetate paper (hospitex Diagnostics) using barbitone buffer (pH=8.6) under 200V for 1 hour in electrophoresis apparatus (Hospitex Diagnostics). Electropherogram was then dried, stained by Ponseu S and quantification of serum protein bands was done by densitometer (Hospitex Diagnostics).

The HbA1c estimation was done on EDTA treated blood samples using Nycocard instrument which follow boron affinity chromatography principle.

Statistic analysis was done using SPSS software 17.0 version. One way ANNOVA was applied for difference in α1 and α2 serum proteins concentration between controlled and uncontrolled DM.

RESULTS

The range of HbA1c in the study was 5.7-14.5%. Table 1 shows the mean and standard deviation of different bands of electropherogram in controlled and uncontrolled type

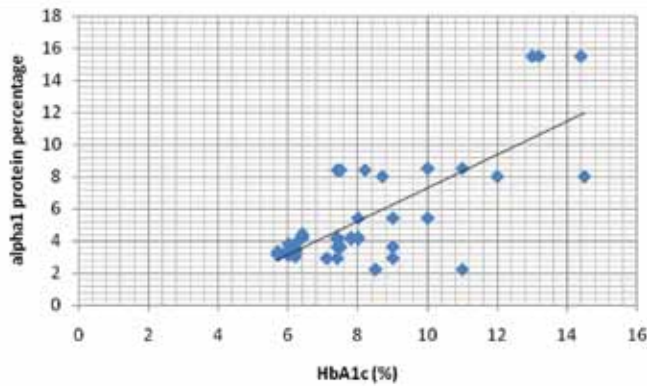


Figure 1A: Relationship of α_1 protein percentage and HbA1c

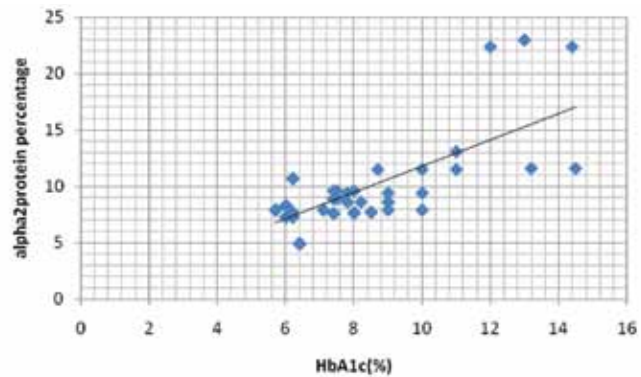


Figure 1B: Relationship of α_2 protein percentage and HbA1c

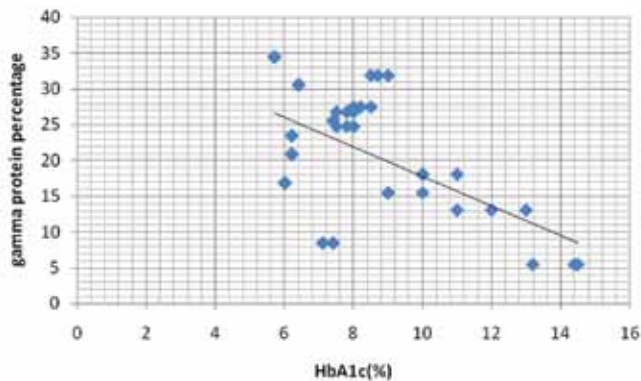


Figure 2: Relationship of γ protein percentage and HbA1c

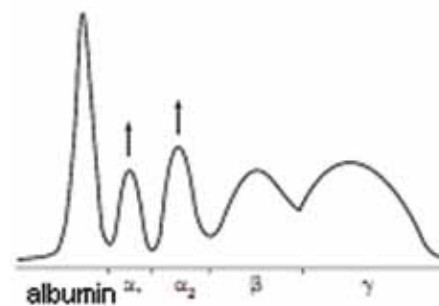


Figure 3: Early phase acute inflammatory pattern showing increase in α_1 (α_1 -glycoprotein and α_1 -antitrypsin) and α_2 (α_2 -macroglobulin, haptoglobin) bands in electropherogram

2 DM. A significant increase in percentage of α_1 - and α_2 -band proteins ($r=0.765$ and $r=0.716$, $p<0.001$) was found with the increasing percentage level of HbA1c indicating positive correlation (fig. 1A&B). γ -band shows significant decrease with increasing percentage level of HbA1c indicating negative correlation (fig. 2).

With cutoff of HbA1c=7 %, the α_1 - and α_2 - serum proteins concentration are significantly higher ($p<0.001$) in uncontrolled DM compared to controlled DM.

DISCUSSION

Serum protein electrophoresis is widely available in university hospital in Nepal. It depicts different patterns of protein bands in response to acute and chronic inflammation, various malignancies, liver or renal failure, and hereditary protein disorders.¹⁰ In this study, serum protein electrophoresis showed significant increase in APPs in uncontrolled DM compared to controlled DM. There was increase of positive APPs, as indicated by increased percentage of α_1 - and α_2 - bands on serum protein electropherogram which is concomitant with early APPs pattern (fig. 3). There is also decrease in γ -band percentage in uncontrolled DM. This shows that in the backdrop of low grade chronic systemic inflammation for the onset of

Type 2 DM, there is tendency towards acute onset of new inflammation everytime if DM goes uncontrolled. In other words, systemic inflammation can definitely be slowed down by glycemic control in DM patients which will slow down the macrovascular and microvascular complications. These findings are in agreement with other authors who worked with APPs in type 2 DM.^{7,11}

This increase in APPs in uncontrolled DM illustrate that inflammatory status deteriorates with increase in HbA1c. Similar report has been concluded by other studies.^{3,5} Advanced glycated end (AGE) products are trigger for inflammatory response. Elevated glucose levels promote inflammation by increasing oxidative stress due to the formation of AGEs and increased TNF.¹² Uncontrolled DM have more glycated molecules which lead to exacerbation of inflammation resulting in increase percentage of APPs. In contrast to our study, an increase in only α_2 - band has been reported by some studies in advanced DM.^{10,13}

Increase in α_1 band percentage is contributed by APPs α_1 -antitrypsin, α_1 -antichymotrypsin and α_1 acid glycoprotein. Similarly, increased α_2 band is due to increase in APPs (ceruloplasmin and haptoglobin). However this study could not detect the CRP band which is said to lie in between β and γ -band.⁹ CRP must be analysed by immunoassay.

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Similarly another APP- fibrinogen couldnot be analysed by this technique as the sample taken was serum. β -band was not increased in this study as transferrin decreases and C3 increases. Both transferrin and C3 lie on the same band region β . This study showed that inflammation status can be screened in DM patients by CAE of serum proteins depicting the early phase acute inflammatory status.¹⁰

CONCLUSION

CAE of serum proteins show early phase acute inflammatory status in uncontrolled type 2 DM. The process of systemic inflammation worsens with uncontrolled glycemia as indicated by HbA1c. Inflammatory status should be studied adjunct to glycemic status. Our study recommends inflammatory status be studied along with glycemic status in diabetic patients.

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