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Original Article

Study of Diabetic Hypertensive Nephropathy in Local Population.

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Abstract

Introduction: Studying the biochemical parameters and levels of protein biomarkers that influence hypertensive diabetic nephropathy in the Pakistani population is the goal of the current research endeavour. The Sheikh Zayed Hospital in Lahore, Pakistan, was used to recruit 50 age- and sex-matched healthy normal controls and 100 diabetic patients with hypertensive nephropathy.

Methodology: Three groups of people were formed, with group 1 being the control, group 2 being the diabetic hypertension without nephropathy, and group 3 being the diabetic hypertensive with nephropathy. Urine from the previous day and blood were taken and kept for later analysis. By using a 2-D liquid chromatographic system and mass spectrometric standard reference methods, biochemical parameters associated with hypertensive diabetic nephropathy and particular protein markers were analysed.

Results: By using MALDI TOF TOF analysis, the proteins that differed between test and control samples were found.According to the biochemical data, the diabetic groups with hypertensive nephropathy had significantly higher values of fasting blood sugar, diastolic and systolic blood pressure, total serum and urine proteins than group 2od without nephropathy and the control group in group 1.

Conclusion: When compared to patients who are normal and do not have nephropathy in the Pakistani population, the levels of proteins that serve as biomarkers, such as albumin, are significantly higher in the diabetic hypertensive with nephropathy group.

Key words: diabetes, population, disease, patient, hospitals

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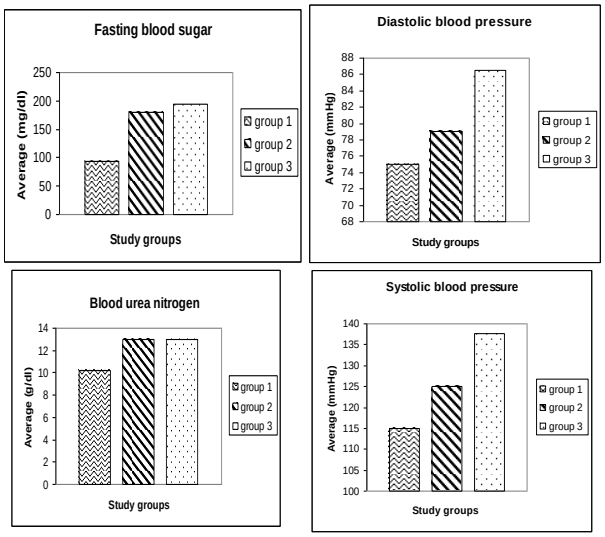
Introduction

Within the next 25 years, diabetes mellitus is expected to rank among the top disablers and deaths in the globe. Pakistan will move up to fourth place in the global diabetes rise. In the absence of basic prevention, the diabetes epidemic will spread. Diabetic hypertensive nephropathy affects an increasing percentage of national health expenditures as its prevalence increases globally.

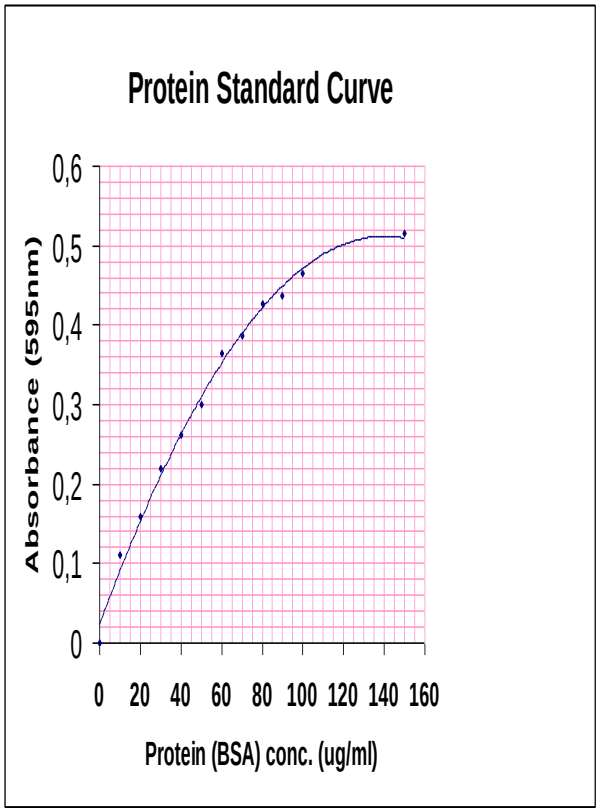
The damage to the glomeruli and tubules caused by diabetes develops over a number of years, and it's probable that protein excretions from the glomeruli and tubules came before macro-albuminuria, possibly even microalbuminuria, and eventually kidney failureProteomic profiling, one of the newest and most sensitive technologies, has the potential to detect urinary proteins linked to the development of diabetic nephropathy long before changes in kidney function or urine albumin excretion are visible clinically



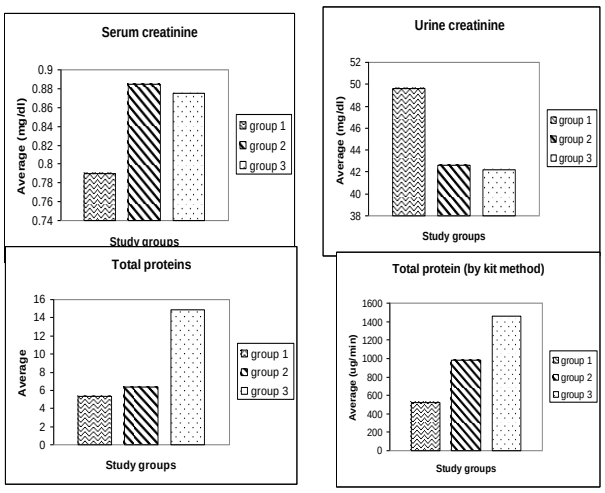
Experimental

The hypertensive nephropathic diabetic patients and sex-matched normal healthy controls of the same age were enlisted from Sheikh Zayed Hospital in Lahore, Pakistan. Three groups of people were created: group A represented the control condition, group B represented diabetes hypertension with nephropathy, and group C represented diabetic hypertension without nephropathy.

By using a 2-D liquid chromatographic system and mass spectrometric standard reference methods, biochemical parameters associated with hypertensive diabetic nephropathy and particular protein markers were analysed. By graphing the standard curve depicted in Figs. 1a and b, the amount of total proteins and albumin excretion rate were measured.



According to the biochemical data, the diabetic group 3 with hypertensive nephropathy had significantly higher levels of fasting blood sugar, diastolic and systolic blood pressure, total serum proteins, total urine proteins, and albumin excretion rate. The non-significant findings in the albumin excretion rate and blood pressure were seen in the other groups, control (group 1) and group 2, as shown in Fig. 2.



Protein Profiling

Fig 1:a)Standard curve for proteins to estimate total serum proteins
b) Micro albuminurea concentration estimation in urine samples.

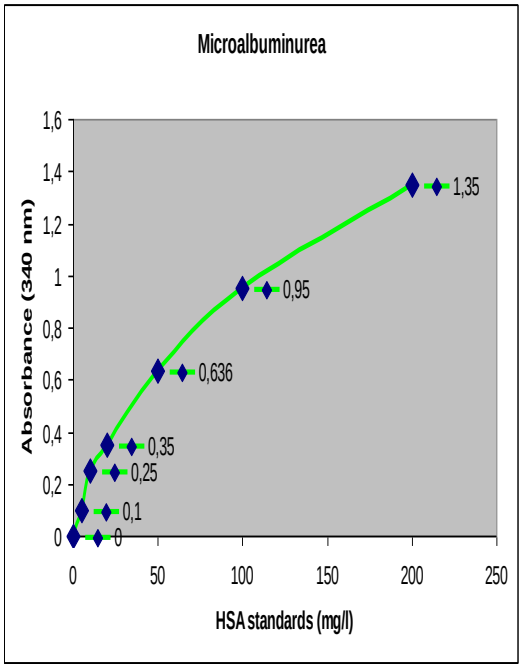


Fig 2:Comparison of all three groups' biochemical relative parameters: group 1 is the control group, group 2 is the diabetic group without nephropathy, and group 3 is the diabetic group with hypertension and nephropathy.

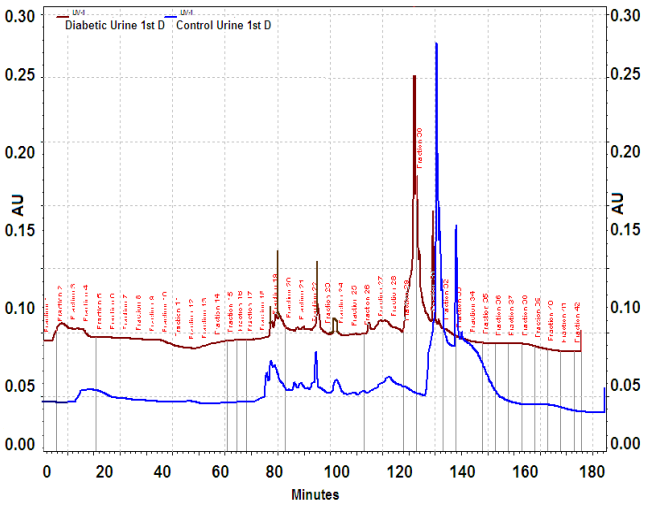
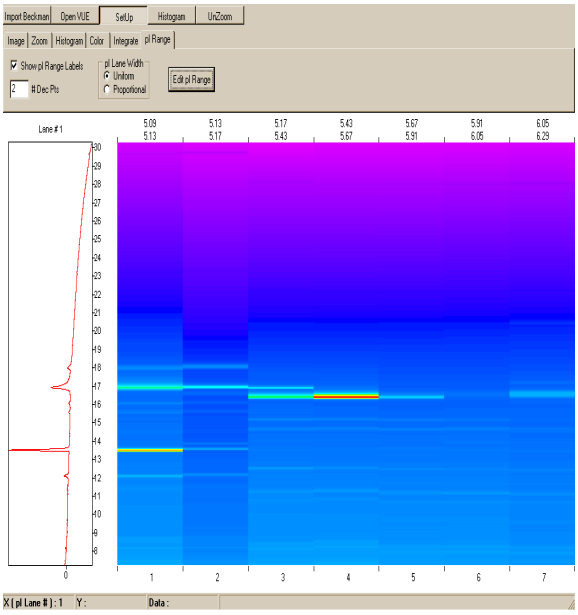


Fig 3:The comparison of control and diabetic urine samples segregated based on

pI values is shown in a 2 D liquid chromatography study.

SDS-PAGE and 2D Liquid Chromatography analysis:

The protein profiling of human urine by SDS-PAGE analysis is shown in Figure 3a. Hypertensive patients have higher levels of albumin than normal in the commassie staining.Albumin is a primary abundant protein that is present or seen in the urine samples of individuals with hypertensive nephropathy; less albumin was seen in the normal control group and in diabetic patients who did not have nephropathy.Figure 3 b. displays the results of a 2D liquid chromatographic analysis and comparison of a control urine sample with a urine sample from a diabetic patient, demonstrating elevated levels of some proteins in the diabetic sample but low expression in the control sample. Proteins were separated and subjected to additional analysis after being identified and purified by chromatofocusing, reverse phase analysis, and protein separation.

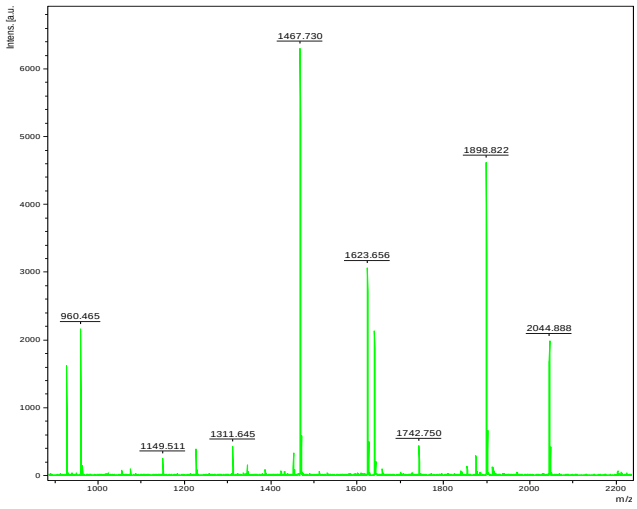


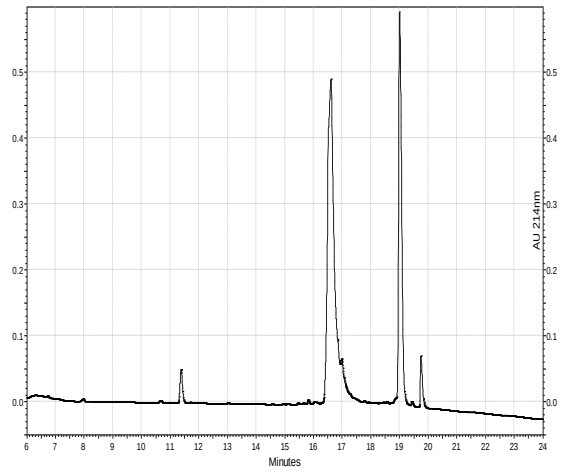
The ProteoVue software imports the data from the 2D liquid chromatography and give the pI map of the all proteins separated into various fractions in the Fig 4a. DeltaVue software compares the both ProteoVue maps of control as in red colour and diabetic as in green colour shows in the Fig 4 b. The prominent peak shows the presence of human albumin in the pI range of 6.38-6.68.

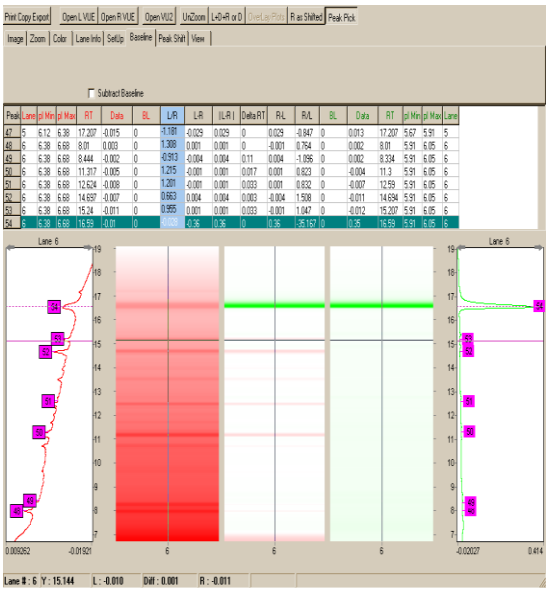
Fig 4:(a) ProteoVue analysis of the fractions separated by ChromatofocusiaChromatofocusiang fractions underwent proteoVue analysis. (b) Delat Visual examination of a chosen fraction reveals higher protein expression in the diabetic sample.

Mass Spectrometric Analysis

The fraction chosen from the two-dimensional analysis was purified and then subjected to further mass spectrometric analysis, as shown in Fig. 5a.Figure 5b displays the intact mass of the human albumin that was determined by MALDI TOF TOF. While the MASCOT analysis, as shown in fig. 5c, corroborated the tryptic digestion of the human albumin protein.







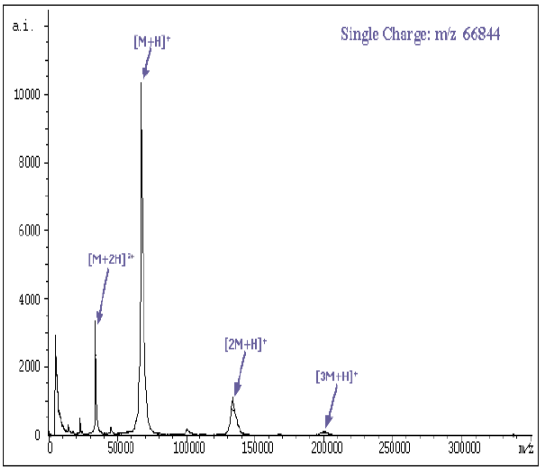


Fig 5:(aAnalysis using mass spectrometry of the albumin-containing fraction of the 2DLC separation. (bHuman albumin in urine samples was typertically digested and its intact mass was determined by MALDI TOF TOF (c) and mass spectrometric analysis.

Conclusion

According to the aforementioned research, the prevalence of diabetic hypertensive nephropathy is significantly higher among Pakistan's local diabetic population than it is among those who do not have the condition. When compared to healthy and diabetic individuals without nephropathy, these patients' albumin excretion rates and levels are much higher. Albumin serves as a protein biomarker for this condition. Therefore, additional study is required in this area in order to manage hypertensive nephropathy in diabetics and avoid

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