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QUALITATIVE ANALYSIS OF SERUM PROTEINS IN BENIGN PROSTATIC HYPERPLASIA SEPARATED BY SDS-PAGE

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ABSTRACT

This study was conducted for qualitative analysis of serum proteins separated by SDS-PAGE and stained by Coomassie Brilliant Blue R-250 in order to describe the preliminary identification of serum proteins that may act as diagnostic marker in benign prostatic hyperplasia. Serum samples of 23 biopsy confirmed cases of benign prostatic hyperplasia and normal controls of similar age group were subjected to SDS-PAGE on a 12% resolving gel, followed by staining with Coomassie Brilliant Blue R-250. Protein fractions were analyzed using computer software program "GeneGenius Gel Documentation and Analysis System". Major protein fractions ranging in molecular weights from 1.45-157 kDa were observed. Raw volumes of most of the protein fractions seem to be increased in majority of benign prostatic hyperplasia cases as compared to normal control. Protein fractions 55-57 kDa were undetected in normal controls under 80 years of age but appeared in 56% of benign prostatic hyperplasia cases. Two dimensional gel electrophoresis and silver staining of these samples could yield better resolution of protein fraction 55-57 kDa that could serve as marker for benign prostatic hyperplasia.

Keywords: benign prostatic hyperplasia, analysis, serum protein, SDS-PAGE.

INTRODUCTION

Benign prostatic hyperplasia (BPH) has been variably defined as prostate enlargement, histologic hyperplasia, lower urinary tract symptoms, diminished uroflow or urodynamic measurements or it has been viewed as an indication for prostatic surgery. The prostate gets larger in most men as they get older, and overall, 45% of men over the age of 46 can expect to suffer from the symptoms of BPH if they survive 30 years [1]. Incidence rates increase from 3 cases per 1000 man-years at age 45-49 years, to 38 cases per 1000 man-years by the age of 75-79 years. Whereas the prevalence rate is 2.7% for men aged 45-49, it increases to 24% by the age of 80 years [1]. Increase in age and an intact androgen supply seem to be prerequisites for BPH development. Apart from that, no other risk factors have been identified uncontrovertibly [2]. The incidence of BPH is far higher than prostate cancer that causes the greatest number of cancer deaths in American males [3].

The PSA serum test has contributed to earlier detection, however, 65-75% of moderately elevated PSA levels are attributed to BPH, often resulting in unnecessary biopsies [4]. Several approaches have been undertaken to improve the PSA test such as measuring PSA velocity [5], PSA density [6], and assessing ratios between free, complexed and total PSA serum values with various degrees of success [7]. Combinations of markers such as free PSA, IGF-I, and IGF-binding protein 3 have resulted in improved diagnostic discrimination between BPH and prostate cancer [8]. Additional improvement in early detection, diagnosis, and prognosis will likely require the measurement of a panel of protein biomarkers.

Proteins initiate all cell functions and pathways, identifying differentially expressed proteins between normal and pathological state, leading to a better understanding of the cellular mechanisms involved in disease. Some proteins are down-regulated and others are up-regulated with the onset of disease, depending on a protein's specific function, undergoing disease-specific posttranslational modifications [9-11]. This identification of changes in protein profile that occur during pathological condition could lead to the discovery of protein biomarkers and novel strategies for the improvement of detection and diagnosis of BPH without any discrimination. This will also minimize unnecessary biopsies.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a versatile and powerful technique widely used for protein separation based on their molecular weights [12]. In this study protein fractions were studied by SDS-PAGE due to its importance in the resolution of serum proteins. In this study the aim of performing SDS-PAGE was to make a comparison between protein profiles found in healthy subjects and BPH cases an to identify some novel proteins that are lost or changed (raised or lowered) in concentrations in diseased subjects.

MATERIALS AND METHODS

This study was conducted at NHRC a centre of PMRC and Institute of Biochemistry and Biotechnology University of the Punjab Lahore.

Inclusion criteria

BPH subjects were selected on the basis of lower urinary tract symptoms (frequent urination, urgency and dribbling) and DRE findings, which were further, confirmed BPH patients on biopsy. Controls displayed none of these symptoms.

Exclusion criteria

Patients who had undergone any surgical intervention of prostate were excluded. Biopsy confirmed prostate cancer patients were also excluded from study.

Blood samples of 23 biopsy confirmed BPH patients of different ages were collected from Urology units of Shaikh Zayed Medical Complex and Jinnah Hospital Lahore. Normal healthy males matched by age were also included from general community as control group. Similarities of their basic confounding factors were ensured. The samples were distributed into four groups according to age as group I (50-59 yrs.), group II (60-69 yrs.), group III (70-79 yrs.) and group IV (>80 yrs.). Group I (50-59 years) included one normal control (N_1) and six BPH cases (B_1-B_6). Group II (60- 69yr.) included one control (N_2) and six BPH cases (B_7-B_{12}). Group III (70-79 yrs.) had one control (N_3) and five BPH cases (B_{13}-B_{17}) and group IV included a control (N_4) and six BPH cases (B_{18}-B_{23}). Sera were separated and stored at -80^{o}C till analysis.

Reagent preparation

12% resolving gel

8mls were prepared by dissolving 30% acrylamide-bisacrylamide, 3.35ml of 3M Tris-HCl (pH-8.8), 0.2ml of 10% SDS in 8.45ml of distilled water, followed by 4ul TEMED and 65ul of 10% ammonium persulfate.

Stacking gel

It was prepared by dissolving 0.9ml of 30% acrylamide-bisacrylamide, 55ul 10% SDS, 0.35ml of 1M Tris-HCl (pH 6.8) in 4.9ml distilled water followed by addition of 38ul bromophenol blue, 5ul TEMED and 55ul 10% ammonium persulfate.

Tris-glycine buffer

15g of trizma base, 72g of glycine and 5g SDS were dissolved in distilled water and final volume was made up to 1000ml. The solution was 5x diluted before electrophoresis.

Staining solution

0.5g Coomassie Brilliant Blue R-250 were dissolved in 450ml methanol and then mixed with 90ml glacial acetic acid and 450ml distilled water.

Destaining solution

It was prepared by mixing 300ml (30%) methanol, 100ml (10%) acetic acid and 600ml water.

Since equal quantities of protein from each sample under comparison must be loaded on to gels for electrophoretic analysis, so the total protein content of each serum sample (cases and controls) was estimated by Bradford assay (1976) [13].

In each group serum samples of control and BPH cases were separated by loading in their respective wells. The gel was then electrophoresed at constant supply of 12mA and voltage of 150V in a minicold lab maintained at 4^{o}C. The gel was stained using Coomassie Brilliant Blue R-250 and then destained. Protein fractions appeared as dark bands on a light background. Gels were photographed using "GeneSnap" and their images were formatted and analysed by using "GeneTools", which were part of the computer software program "GeneGenius Gel Documentation and Analysis System". The molecular weight of each protein fraction was determined by using molecular weight markers as standard. The raw volume was calculated by the software using band height and intensity, which was a measure of relative quantity of protein in each sample.

RESULTS

In group I (50-59 years) protein fractions ranging in molecular weight from 1.45-157 kDa were observed when samples were subjected to SDS-PAGE. Raw volume of most of the protein fractions in this group were increased in majority of BPH cases, while some proteins were decreased as compared to normal control (N_1). In one sample (B_5), it was observed that most of the major protein fractions (157, 140, 131, 122, 114, 100 and 76) were undetectable and remaining was decreased in raw volume when compared to other cases and normal control in same group. Furthermore raw volumes of all protein fractions were also down regulated in sample (B2). Protein fractions 55-57 kDa molecular weights were also found absent in the control but present in two of BPH cases (B_4, B_6). (Figure-1)

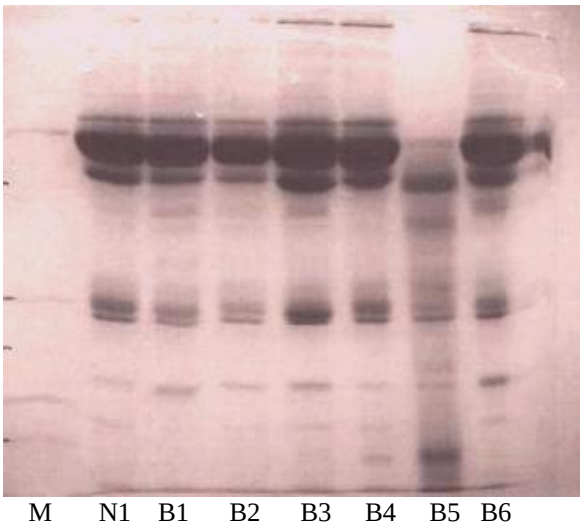
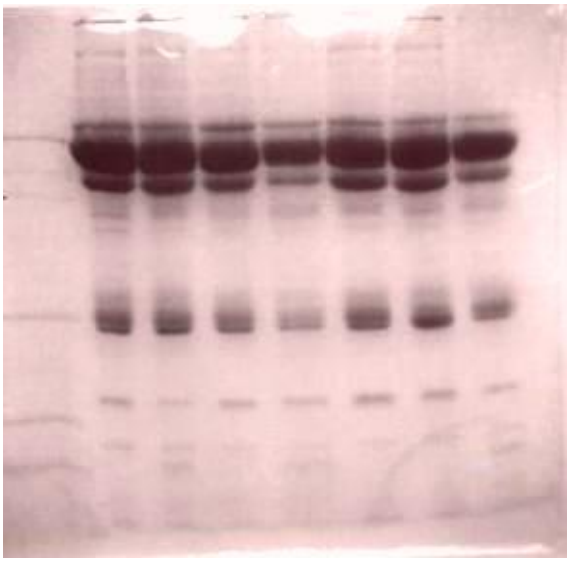


Figure-1 Serum protein profile of control (N_1) and cases (B_1-B_6) in group I (50-59 yr.), resolved on 12% resolving Gel by SDS-PAGE.

M: Protein size markers (from top to bottom): 67, 45, 24, 18, 13 and 1.45 kDa.

In group II (60-69 years) when samples of BPH cases and normal control were subjected to SDS-PAGE, it showed same patterns as in group I. Raw volume of protein fractions were up regulated. Exceptional behavior was observed in two samples (B_9 and B_{12}), in which protein fractions of molecular weight 131, 122, 114 and

100 kDa, appearing in all the other samples were absent and remaining fractions were decreased in raw volumes. Like group I, protein fraction of 55-57 kDa was also found absent in control (N_2), but it was seen in BPH cases i.e. B_7, B_8 and B_{10} of group II (Figure-2).

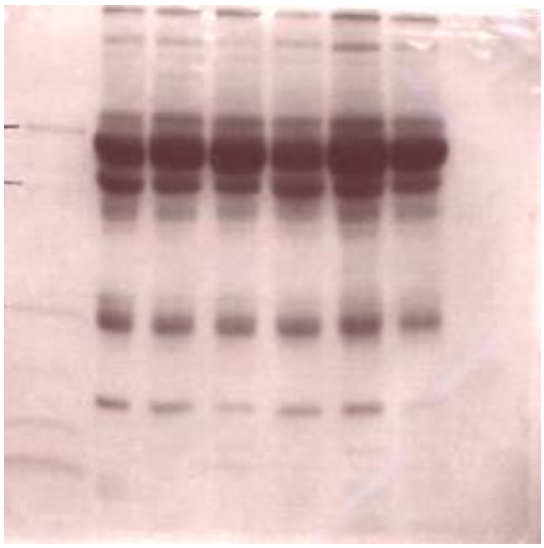


M N2 B7 B8 B9 B10 B11 B12

Figure-2. Serum protein profile of control (N2) and cases (B_7-B_{12}) in group I (60-69 yr.), resolved on 12% resolving gel by SDS-PAGE.
M: Protein size markers (from top to bottom): 67, 45, 24, 18, 13 and 1.45 kDa.

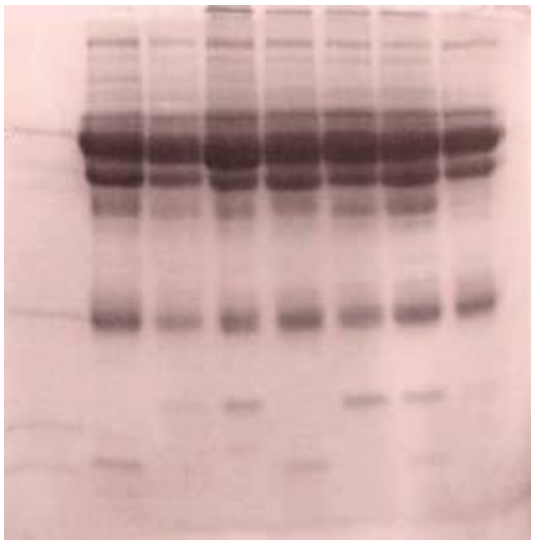
Unlike group I and II, group III (70-79 years) showed an overall increase in raw volume of protein fractions in all BPH cases (B_{13}-B_{17}) as compared to normal control (N_3). Whereas similar to normal controls in group I and II protein fractions 55-57 kDa was absent in the control (N_3) but appeared in B_{15} and B_{16} BPH cases in this group (Figure-3).

In group IV (>80 years), the raw volume of most of the protein fractions was found to be increased in BPH cases relative to the control (N_4). Anomalous behavior in this Group was displayed by one sample (B_{23}), in which four protein fractions of molecular weights 131, 122, 114 and 100 kDa were not seen. The 114 kDa fraction was also found absent in two samples (B_{18} and B_{22}). Protein fractions 50-57 kDa was seen in control (N_4) of this group which was found absent in normal controls of all other groups. These protein fractions were also seen in all BPH case (B_{18}-B_{23}) of this group (Figure-4).



M N3 B13 B14 B15 B16 B17

Figure-3. Serum protein profile of control (N_3) and cases (B_{13}-B_{17}) in group I (70-79 yr.), resolved on 12% resolving gel by SDS-PAGE.
M: Protein size markers (from top to bottom): 67, 45, 24, 18, 13 and 1.45 kDa.



M N4 B18 B19 B20 B21 B22 B23

Figure-4. Serum protein profile of control (N4) and cases (B_{18}-B_{23}) in group I (> 80 yr.), resolved on 12% resolving gel by SDS-PAGE.
M: Protein size markers (from top to bottom): 67, 45, 24, 18, 13 and 1.45 kDa.

DISCUSSIONS

Benign prostate hyperplasia or benign prostatic hypertrophy (BPH) is an enlargement of prostate gland as man ages. Role of androgens seem to be prerequisites of BPH. Protein biomarkers are very useful for diagnosis and prognosis of various forms of cancers and other diseases. Certain proteins can be up or down regulated during disease progression. Detection of these differences in protein expression levels, as a function of disease

progression is important for prognosis. To distinguish between normal and pathological state of benign prostate hyperplasia, a qualitative analysis of Coomassie Brilliant Blue-stained proteins separated by SDS-PAGE was undertaken in this study. This procedure yielded 14 major protein fractions ranging in molecular weight from 1.45-157 kDa, their appearance and raw volume in serum sample of each case was, studied (Figures, 1 to 4)

On the whole, it was observed that raw volume of most protein fractions was increased in majority of BPH cases as compared to controls. In the present study protein fractions 55-57 kDa appeared in 56% of BPH cases (B_4, B_6, B_7, B_8, B_{10}, B_{15}, B_{16} and B_{18}-B_{23}) and was undetectable in normal controls under the age of 80 years. Similar findings were reported by other workers, who compared subcellular proteins of normal, BPH and prostate cancer cases using SDS-PAGE. They found significantly higher concentrations of non-histone proteins of 42 KD, 55KD and 190 KD in BPH cases than in the normal controls and cancer prostate, their results also showed that contents of other major protein species were similar in specimen of normal and diseased prostate cases [14]. Serum and not tissue samples were used in the present study to compare the protein profiles among study groups. Another study showed that 56 kDa protein fraction was up-regulated in 86% of BPH cell lysate than prostate cancer and normal controls [15].

Previous studies have shown that the concentrations of most of the proteins are too little to be detected by Coomassie Blue staining. However, these proteins could not consider “unique” to their respective samples, because amount of proteins that did not exceed the minimum sensitivity of the dye may have been present in other samples [16]. In the present study, SDS-PAGE instead of 2D-PAGE and Coomassie Brilliant Blue R-250 in place of silver staining was used; therefore, amount of protein fractions seen was much lesser. As quoted above, the protein fractions of 55-57 kDa, which were undetectable in the controls N_1, N_2 and N_3 and was present in their corresponding BPH cases, cannot be considered totally absent in these controls as well as other BPH cases, because amounts of protein that did not exceed the minimum sensitivity of the dye could be present.

In another study where serum proteins of prostate cancer were separated by SDS-PAGE, it was shown that protein fractions of molecular weights 1.27, 100, 114, 122, and 140 kDa were absent in significant number of prostate cancer cases [17]. In the present study also such an inconsistent behavior was shown by sample B_2, B_5 (Group I), B_9, B_{12} (Group II) and B_{23} (Group IV), in that major protein fractions were either undetectable or down regulated in them. This might possibly due to some ailment other than prostate enlargement, since BPH cases were not necessarily clear of other health complications. Protein fractions of molecular weights 131, 122, 114 and 100 kDa were absent in B_5, B_9, B_{12} (Group II) and B_{23} (Group IV). Furthermore, 114kDa protein fraction was also found absent in B_{18} and B_{22} (Group IV). These results might be evident of their malignant transformation and need to be reassessed by other methods.

CONCLUSIONS

Further investigation of these samples could yield more information; silver staining, which is more sensitive than Coomassie Blue, would tell whether minute quantities of these protein fractions were present. Two dimensional gel electrophoresis would separate the proteins according to ionic potentials as well as molecular weight and thus achieve better resolution. Comparison of serum protein profiles in prostate cancer and benign prostatic hyperplasia along with normal controls can give an insight for the improvement in detection and diagnosis of BPH without any discrimination and unnecessary biopsies.

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