Original Article

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Profiling of SDS-PAGE Based Differentially Expressed Proteins from Sera of Patients with Prostate Carcinoma

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ABSTRACT

Objective: This study "Profiling of SDS-PAGE based differentially expressed proteins from sera of patients with prostate carcinoma" provides an update on the novel candidate biomarkers in development, which have shown potential for improving the detection of clinically significant cases of this malignancy. **Study Design:** Cross sectional studies. **Settings:** Bolan Medical Complex and Civil Hospital Quetta **Duration:** From 08-01-2018 to 30-06-2018. **Methodology:** In this study, 50 serum samples were screened from prostate cancer patients and 10 from healthy donor sera using SDS PAGE in order to investigate the protein expressions in the form of bands. We compared these proteins with controls of healthy donors to find their upregulation **Results:** The results reveled the upregulation of proteins found in Prostate Cancer Patients as compare to healthy donors which were used as a control, which can further used as a biomarker for the diagnosis of early stage Prostate cancer. **Conclusion:** In the study we initially found 33 kDa, 34 kDa, 50 kDa, 75 kDa, and 100 kDa to be candidates for PCa progression biomarkers in Prostate cancer serum patients.

Keywords: Prostate cancer, SDS PAGE, Biomarkers.

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INTRODUCTION

Prostate cancer is most prevalent in men and worldwide is the second most widespread malignancy detected. It plays a key role in cancer related mortality and morbidity.[1] Worldwide the cases of prostate cancer being reported have different incidence rate. The observed findings showed that South Asia has low incidence rate and North America has the highest observed incidence rate.[2] Due to the treatment and diagnostic procedure the incidence rate of Prostate cancer varies among different areas.[3]

In US and Europe the PCa is termed to be in third position for causing deaths and at second position for being the most commonly diagnosed cancer.[4] In Asia around 107.2 /100,000 incidence of prostate cancer occurred, which are considered as lowest. While in America and Africa the incidence rate were highest i.e. 275.3/100,000.[5] During the year 2016, the reported cases of death from PCa were 26,120 and identified cases were 180,890.[6]

At early stage Prostate cancer remains asymptomatic, the patients of PCa represent the advanced diseases like bony pain, hematuria, urinary tract symptoms and pelvic pain. Sometimes prostate cancer effects the bone mineral density of the patients[7] and symptoms in metastatic prostate cancer are relieved when short course of radiotherapy is given to such patients.[8] Without performing the diagnosis tests the male patients of PCa cannot diagnose this disorder.[9] The prognosis and therapeutic drug selection of the disease become easy with the emergence of markers.[10]

Prostatic epithelial cells secret a 34-kDa 240-amino-acid glycoprotein called PSA (Prostate-specific antigen).[11-13] PSA is a known diagnostic biomarker for calculating the Prostate cancer risk.[14-15] Prostate specific antigen is a biomarker approved by FDA for prostate cancer diagnosis.[16] PSA is a serologic biomarker that allows to detect PCa from suspected patients.[17, 18]

PSA sheds in the blood. Prostatic biopsy is recommended for the PCa patients having the (4.0 ng/mL - 10.0 ng/mL) PSA serum volume.[19] Over diagnosis is the main issue with this marker because its level can rise in non-cancer diseases which makes it less specific.[10]

Benign Prostatic Hyperplasia (BPH) is the non cancer condition where the PSA level rises which makes PSA to give false negative and false positive results.[20]

Molecular Biomarker leads to the identification of new therapeutic products and helps the pathological stage of disease to be well predicted.[21] For the improvement of PSA diagnosis new molecular biomarkers are required, which can also help in predicting and improving the possibility of disease development.[20]

For discovering advanced biomarkers proteomic methods play vital role.[22] For human protein Quantitation the precise method known is (two dimensional difference gel electrophoresis) 2D-DIGE. This method helps in the analysis of small sample amount on gel and improves the integral variability.[23, 24]

Some studies have shown an over-expression of many tumor-associated antigens (TAAs), and the production of autoantibodies in TAAs can be used as a diagnostic biomarker. The aim of the present study was to predict the candidate biomarkers responsible for characterizing the presence of diseased or pathological conditions.

METHODOLOGY

Study Design: Cross sectional study.

Settings: Bolan Medical Complex and Civil Hospital Quetta Pakistan.

Duration: From 08-01-2018 to 30-06-2018.

Sample Size: 50 Cases.

Sample Collection: From PCa positive patients 50 serum samples were obtained and a pool of 10 sera was taken from 10 healthy donors as control, all samples were taken from Bolan Medical Complex and Civil Hospital Quetta. Falcon tubes without EDTA or anticoagulant were used for collecting the samples. For separation of serum blood samples were centrifuged for 5-10 minutes at 12000 rpm and stored at -80°C.

SDS PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique was used for the separation of proteins on the basis of their molecular weight. Two types of gel (resolving and stacking gels) were used with concentrations as given:

Stacking gel (polyacrylamide 4%), (30%) Acrylamide, (0.8%) Bisacrylamide, 0.5M Tris with pH =6.8 (Alfa Aesar), 10% SDS (Amresco), 0.05% TEMED (Thermo Scientific™), 10% Ammonium persulfate (TR International, Inc.-seattle, WA).

Resolving gel (polyacrylamide 12%), 1.5MTris pH =8.8 (Alfa Aesar) 10% SDS (Amresco), TEMED 0.05% (Thermo Scientific™), 10% ammonium persulfate (TRInternational, Inc.-seattle, WA). For visualization of protein bands Comassie brilliant blue (Thermo Scientific™) staining was used and images of stained gel were observed using Transilluminator apparatus (Labnet).

Coomassie Blue Staining: Proteins separated by SDS-PAGE were visualized by staining the polyacrylamide gels. Coomassie Brilliant Blue (CBB) staining technique was used for this purpose. In spite of the wide dynamic range of various fluorescent detection methods and high sensitivity of silver staining, staining with Coomassie Brilliant Blue remains the most widely used technique for detecting the separated proteins electrophoretically.[25, 26] CBB staining was originally developed for staining the proteins on a sheet of cellulose acetate in 1963.[27] The advantages of (CBB) staining are as followed: low cost, ability to change for fast or very delicate staining, visual inspection, ease of use, convenient scanning method for image acquisition and better quantitative analysis of silver coloring. The stain Coomassie has become the most important component of Blue native PAGE over the previous 10 years.[28]

RESULTS

This study was conducted to separate several proteins by SDS-PAGE from prostate cancer sera compared to control group. The differential expression of proteins was observed by screening 50 serum samples from prostate cancer patients and 10 from healthy donors (as controls).

Results revealed that the bands of 33, 34, 50, 75 and 100 kDa were differentially stained by PCa patients sera as compared to control sera (Fig 1). We also observed different heavy bands, two bands of 33 and 34 kDa were seen in 60% (n=30) of PCa patient's sera. A thick band of 50 kDa was observed in 74% (n=37) patients sera which was not observed in staining pattern of control sera. A band of 75 kDa molecular weight was also observed in the staining pattern of 46% (n=23) of PCa patients testing sera. This band was not observed in the staining pattern of control sera. Relatively low intensity band of 100 kDa was stained by 58% (n=29) testing sera and no band of this molecular weight was stained by the pool of control sera.

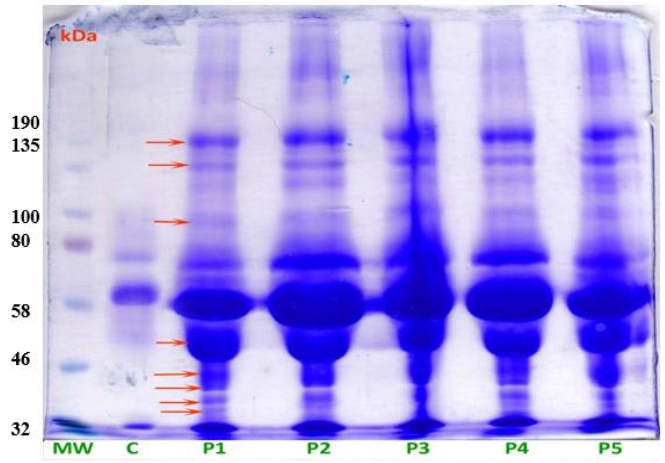

Figure 1: Representative CB staining gel illustrates staining pattern of differentially expressed serologic protein bands (depicted by red arrows) of prostate cancer patients comparing a pool of 10 control sera. 'MW' indicates molecular weight marker 'C' stands for pool of control sera while 'P1-P5' show prostate cancer patient's sera.

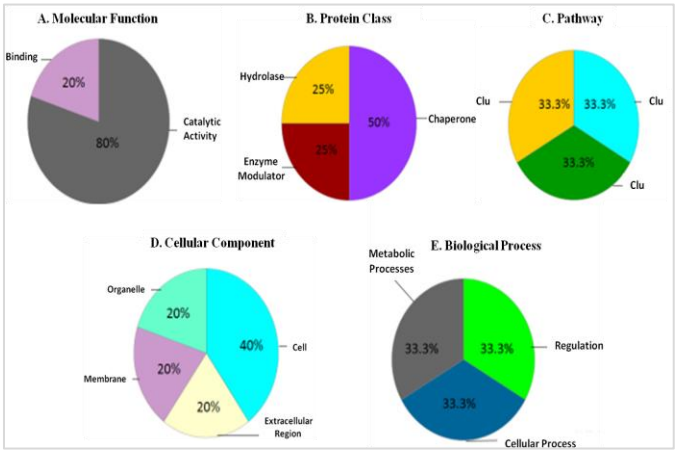

Figure 2: Gene Ontology classification of proteins according to their A. Molecular Functions, B. Protein Class, C. Protein Pathway, D. Cellular Component, E. Biological Process using PANTHER classification

Gene Ontology Analysis: PANTHER (pantherdb.org) Classification System was used to perform Gene ontology analysis for the classification of proteins in order to get high throughput analysis. The classification of proteins was done according to the cellular component, protein class, molecular functions, protein pathways and biological process.

In molecular function category, 80% proteins involved in catalytic activity and 20% proteins were binding proteins. In protein class category, 50% proteins were chaperone, 25% proteins were hydrolase and remaining 25% proteins were enzyme modulators. In protein pathway category, PANTHER classification system identified 3 signaling pathways all carries 33.3% Clu. In cellular component category, 40% were cells, 20% were organelles, 20% were membrane proteins and remaining 20% were extra cellular region proteins. In biological processes category, 33.3% proteins were involved in metabolic processes, 33.3% proteins were involved in cellular processes and remaining 33.3% proteins were involved in regulation processes.

DISCUSSION

The SDS-PAGE analysis of prostate cancer sera revealed the staining pattern of protein bands, with different intensities, which were compared with control sera. Bands of 33 and 34 kDa were observed in our results, these bands might correspond to Prostate specific antigen (PSA). Comparing sensitivity of PSA (40% and 100%), TRAP1 (1.8% and 48%), NGEP (7.7% and 70.3%), CLUSTERIN (4.6% and 56%), which reveled PSA has highest sensitivity in early and advance stage of prostate cancer. Prostate specific Antigen (PSA) is well known biomarker for the Risk prediction, diagnosis and monitoring of prostate cancer.[13-15] PSA is also known as (hk3) human kallikrein, which is a 33-34 kDa protein. It belongs to the family serine protease and first identified in 1970s.[29] It was identified in human sera by Wang *et al*. and then in prostate tissue by Papsidero *et al*.[(23)] The normal neoplastic and hyperplastic prostate tissue produces the PSA. Whereas, a1-antichymotrypsin and a2-macroglobulin, are two types of protease inhibitors where the PSA bounds while others remain unbound and few circulates in serum. Processes like hyperplasia, neoplasia and inflammation, of prostate cause disruption of physiological barriers and rise basal membrane permeability, thereby increasing circulating PSA release.[24]

A 50 kDa band was also stained by PCa patient's sera. CDC37 is a 50 kDa chaperon protein, it's over expression leads to prostate cancer.[25] Further confirmation of this protein is desired through western blotting. Thick bands of 50 kDa were stained in our results. The increased expression of prostate cancer has been observed in a chaperon protein called as Cdc37. Its gene codes 50kDa protein that targets to the molecular chaperone HSP90 of oncoproteins i.e. Cdk4, Src and Raf-1. Cdc37 involved in increased manifestation of premalignant lesions and prostate associated tumors.[26] Reportedly it has been observed that the uncontrolled proliferation is promoted by the expression of this protein which can lead to cause prostate cancer.[7]

A 75 kDa protein band was stained by 46% of our PCa testing sera. TRAP1 is a 75 kDa protein , it is a member of the family of heat shock protein 90 (HSP90), one of the few that plays a role in cancer, neurodegeneration, and other outwardly irrelevant diseases with major but non-exclusive mitochondrial localization.[21] TRAP1 has been described for the first time as a TNF receptor-associated protein.[22] During mitosis it's a chaperone of retinoblastoma protein and a factor that stabilizes cyclophilin 40 (CypD) after heat shock.[13] The permeability transitions, apoptosis and, pore opening is prevented by it.[11] TRAP1 levels are associated in some malignancies, like glioblastoma, cancer of the colon, lung, breast, prostate, and also associated with drug resistance.[23] Studies have focused on prostate cancer. Both TRAP1, and the mitochondrial localization of all HSP90s, are universally expressed in metastatic prostate cancer and localized cancers but unnoticeable in benign prostate hyperplasia or normal prostate. Independent enhanced apoptosis inhibits TRAP1 prostate cancer androgen, the selective targeting of Hsp90 mitochondrial protein (Gamitrinibs) from prostate cancer cells.[24] Nevertheless, prostate cancer was not the only tumor to be investigated for playing part in cancer development of TRAP1. Primary assessment of the expression of TRAP1 in limited number of human colorectal carcinomas showed positive regulation in 65% of cases compared to normal regulated peritumoral mucosa.[25]

NGEP which is also termed as TMEM16G gene is a 95-100 kDa protein located on chromosome no 2 at 2q37.3. The NGEP mRNA constitute of two spliced types. Smaller and a larger transcript encoding 179 and 933 amino acid. The smaller transcript protein (NGEP-S) codes the cytoplasmic protein, and the larger transcript protein (NGEP-L) codes for polytopic membrane protein. According to the RNA analysis NGEP is only spotted in all prostate samples of (BPH benign prostate hyperplasia, Cancer and normal), representing it as a differentiation antigen found in normal prostate and expressing itself in cancers.[14]

We also observed 100 kDa protein band which might corresponds to Clusterin which is a stress protein of 100 kDa which was first identified for forming clustering of different cell types.[16] The gene encodes a chaperon protein termed as, apolipoprotein J, sulphated glycoprotein-2 or testosterone-repressed prostate message-2. It is a glycoprotein having heterodimeric disulphide-linkage and is found in all tissues and human fluids.[26] It has been reported to be involved in physiological processes leading to carcinoma and apoptosis of cell, also in DNA repair, tissue remodeling, cell cycle regulation, membrane recycling, complement regulation and lipid transportation.[27] The over expression of this protein lead to multiple human malignancies involving prostate, kidney, colon cancer and breast cancer.[28] Low levels of CLU have been reported in hormone-naive tissues and increased levels are seen after hormonal therapy.[29]

The results showed the differential expression of proteins which can be further analyzed by dot blotting, ELISA and western blotting techniques. On the basis of SDS-PAGE the proteins were separated in the presence of β-mercaptoethanol.

CONCLUSION

In the study we initially found 33 kDa, 34 kDa, 50 kDa, 75 kDa, and 100 kDa protein bands to be candidates for PCa progression biomarkers in the sera of Prostate cancer patients. The study of serum concentrations of these biomarkers could deliver a noninvasive method to explain the clinical use of serum

biomarker detection in a variety of diseases. Development of new biomarkers is needed to further evaluate the clinical parameters. Although it is necessary to discover new biomarkers and confirm already identified markers during coming years.

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CONFLICT OF INTEREST

There is no conflict of interest involved.

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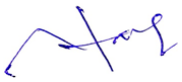
Dr. Muhammad Shahzad Farooq Professor, Department of Biochemistry, Faisalabad Medical University, Faisalabad, Pakistan	Data analysis and review the paper	
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