SEROLOGIC DETECTION OF PROTEINS ASSOCIATED WITH RHEUMATOID ARTHRITIS PATIENTS BY SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

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ABSTRACT

Rheumatoid arthritis (RA) is a systemic autoimmune disease that causes severe join problems. Adults suffering from RA are about 0.5-1% throughout the world. In serum, synovial fluid and other body fluids of RA positive patients, the presence of autoantibodies can be used as diagnostic biomarkers. Number of proteins has been expressed differently during the disease process against which the production of antibodies has been documented to date with diverse sensitivity and specificity. The study was designed to profile differently expressed proteins from sera of patients suffering from RA by SDS-PAGE. Separation of proteins was carried out by using SDS-PAGE and polyacrylamide gels were stained through Colloidal Coomassie Blue staining technique. Protein bands were visualized via transilluminator. Proteins expression of thirty five (male, n=11 and female, n=24) patient's sera have been compared with twenty sera from healthy donors as control. Up regulation of 68 kDa protein was observed in 80 % sera, 50 kDa protein was up regulated in 77% sera of RA patients. Many bands of different molecular weights between 100 and 220 kDa have also been stained in patient's sera but these bands are not documented to date.

Key-words: Rheumatoid arthritis, autoantibodies, biomarkers, autoimmune disease, rheumatoid factor, SDS-PAGE.

INTRODUCTION

Rheumatoid arthritis (RA) is the widespread inflammatory joint disease, people distress from RA is approximately 0.5-1% all over the world (Suresh, 2004; Tamsin and William, 2010) early recognition and management is crucial to stop joint damage and lifelong disability (Catalina and Nancy, 2006). There are no definite standards for early RA diagnosis and treatment. The criteria to deal early RA were given by American College of Rheumatology and the European League against Rheumatism (ACR/EULAR) (Aletaha *et al*., 2010). While Rheumatoid Factor (RF) plays an essential role in the differential diagnosis of polyarthritis because that make it possible to identify RA patients. Thus, RF testing has been one of the classification criteria for RA (Miller *et al*, 2013). RA is a severe illness of bone and cartilage; it causes cruel pain and swelling in joints like knee, wrist, shoulder and hip joints resulting in synovitis (Suresh, 2004). Diagnosis rate of RA victim is 40% within six months after onset of the disease and 70% within two years of the onset of disease resulting in increased bone erosion (Catalina and Nancy, 2006). The exact cause of RA has not yet been identified. This autoimmune disease is more common in women as compare to men by 3: 1 ratio, the genetic and somehow the environmental factors like transmittable agents and smoking may play a vital role in causing this disease (Gabriel, 2001).

The clinical signs, serologic markers and radiographic proof of joint damage are cornerstone to identify RA positive cases (Hoving *et al*., 2004). Rheumatoid factor (RF) is known as early diagnostic marker (Avouac *et al*., 2006). In RA patients sensitivity of RF is 80 % and specificity is about 85%, while antibodies against Cyclic citrullinated peptide (CCP) has high specificity of 96% and low sensitivity of 60–80% (Avouac *et al*., 2006). RF is the well known diagnostic marker that indicates the presence of synovitis in about 75% of the patients (Martinus *et al*., 2002).

Approximately 80% of the patients suffering from joint inflammation are recognized with Anti-citrullinated protein autoantibodies "ACPAs" (Watad and Amital, 2016). The Anti- RA33/A2 antibodies with 33 kDa also serve as diagnostic marker (Steiner *et al*., 1992). RA33/A2 protein is found in 36% of RA positive individuals (Martinus *et al*., 2002). The heavy chain binding protein (BiP) autoantibodies play an essential role in detecting RA victim, the autoantibodies BiP is secreted against p68 protein (Blass, 2001).

A newly introduced novel autoantigen Glocose-6-phosphate isomerase (GPI) in 64% of patients was also helpful in analyzing the joint inflammation; increased titer of anti-GPI was also seen in synovial fluid(Schaller, 2001).The presence of the autoantibodies, anti perinuclear Factor (APF) acknowledged the individual with Rheumatoid Arthritis. APF observed in 71% of the RA patients with 88.5% sensitivity and86.8% specificity (Zeinab *et al*., 2015). Another autoantigen named keratin was also up regulated in RA patients and the production of antibodies against this antigen were produced with 97% specificity, consequently antikeratin antibody (AKA) is a specific serologic biomarker of RA diagnosis (Vasiliauskiene *et al*., 2001).

Anti Sa antibodies with the molecular mass of 50 kDa are highly observed antibodies against Sa antigen in serum samples of 40% RA patients with 37% sensitivity and 98% specificity, somehow Sa antigen looks like same to citrullinated vimentin, Sa antigen located in synovial fluid as well as placenta (Erik, 2004).The autoantibodies including antikeratin, anticitrullinated peptides, anti RA33, anti Sa and anti p68 have 90% specificity for RA (Gunter and Josef, 2002).

MATERIAL AND METHOD

Sera study

Thirty five blood samples were taken from RA positive patients (male, n=11 and female, n=24 of mean age ± 40 years) in addition to the pool of twenty serum samples from healthy subjects as negative control from Akram Hospital Zarghoon Road, Quetta. Unique identification numbers were assigned to each sample in order to retain patient anonymity. The written consent was taken from patients and healthy donors for the use of their sera in this study. Diagnosis of RA positive cases was carried out at Akram Hospital Quetta according to the Rheumatoid Arthritis Classification Criteria 2010, this criteria has been jointly approved by the Board of Directors of the American College of Rheumatology (ACR) and Executive Committee of the European League against Rheumatism (EULAR) (Aletaha *et al*., 2010). And RF test was performed by using RF-Turbi latex kit (Merck France) as per manufacturer instructions. Aliquots of RA positive sera samples were made in sterile eppendorf tubes which were transported to Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta maintaining cold chain and were stored at -80°C.According to the standard protocol the protein amounts were measure by using the BCA protein kit (Bioworld GE Healthcare).

SDS- PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by using electrophoretic unit (cleaver scientific Ltd.) for separating proteins from the serum samples as defined by Ni D *et al*., 2016. Proteins were separated according to their molecular weight using discontinuous ionic buffer system and different ions of two superimposed gels, 04% stacking gel having polyacrylamide 30%, 0.5 M Tris25% (MP biomedicals, LLC), SDS 10% (Invitrogen), TEMED 0.05% (Alpha Aesar), Ammonium persulfate10% (MP biomedicals, LLC) and 12%migration gel having polyacrylamide 30%, 1.5M Tris 25% (MP biomedicals, LLC), SDS 10% (Invitrogen), TEMED 0.05% (Alpha Aesar), ammonium persulfate10 % (MP biomedicals, LLC).The subsequent gels were stained by using Coomassie Brilliant Blue, as described by Merril, 1990 Transilluminator, (WEALTEC dolphin-view), was used to view these gels.

RESULTS AND DISCUSSION

Regarding autoimmune disorders, the detection of disease-specific proteins as autoantigen is critical to considerate their pathogenesis in order to suggest them as a potential candidate for diagnostic and prognostic markers in screening of early phases of autoimmune diseases (Pedotti *et al*., 2003). Among autoimmune diseases, rheumatoid arthritis provides a broad spectrum view with an extremely variable and capricious course of disease development. So, there have been numerous clinical strictures developed to screen RA along with certain clinical parameters. Few RA specific biomarkers such as RF, GPI and CCP are also developed (Korganow *et al*., 1999, Schaller *et al*., 2001) with different specificities for RA diagnosis. It is indubitable that specific biomarkers are

prime need for early diagnosis of RA. Early treatment in RA is important as it can prevent irreversible damage of the joints. In present study sera samples of patients suffering from RA and a pool of sera from healthy volunteers were analyzed by SDS-PAGE.

Thirty five serum samples from RA patients were used to observe the migration pattern of different proteins expressed during the diseased process and compared with a pool of 20 control sera from healthy subjects. The electrophoretic migration pattern among all patients' sera showed up-regulation of certain proteins. An over expression of 68 kDa protein was observed in 80 % (28/35) of sera samples (Fig. 1). This protein was first described as glycoprotein of 68 kDa molecular weight by Blass *et al*, in 1995 with 99% specificity in RA patients and higher percentage of autoantibodies were also observed against this protein (64% sensitivity). Another study also indicated the increased expression (with 66% sensitivity) of this 68 kDa glycoprotein in RA patients (Specker *et al*., 1997). In these studies the autoantibodies were detected against 68 kDa protein by using synovial membrane as an antigenic extract while we had seen only the expression of this protein as clusters by using patient's sera, we did not use synovial membrane or any other body tissue.

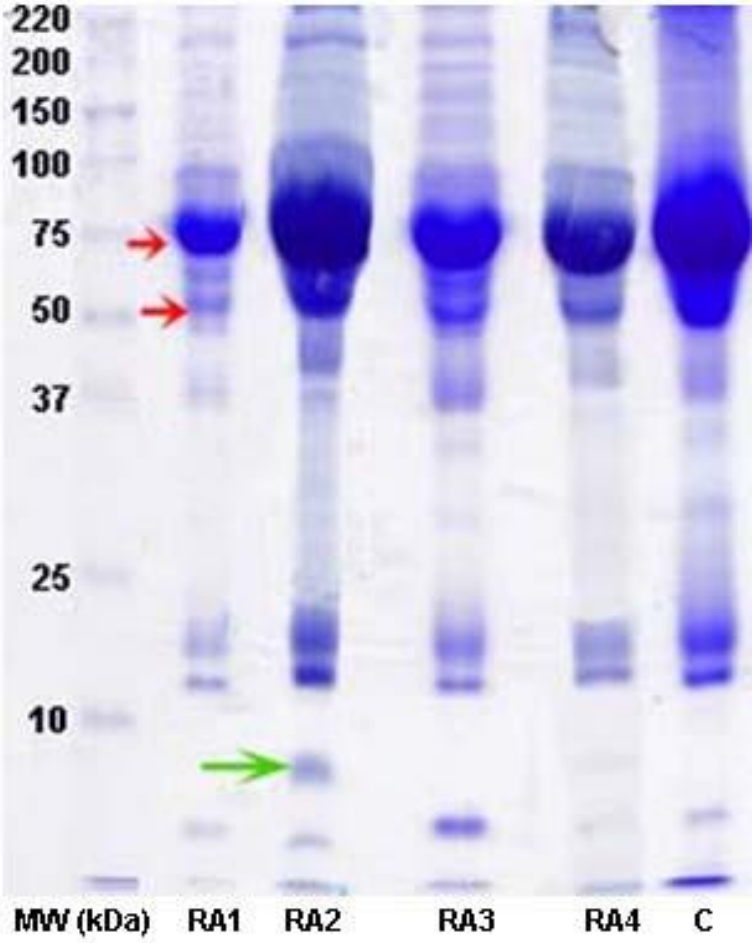

Fig.1. Representative gel of serum samples from RA diagnosed patients and pool of twenty sera from healthy subjects were processed and run on SDS-PAGE for the profiling of differently expressed proteins. From left to right; protein ladder for molecular weight (MW), RA1, RA2, RA3, RA4 are Rheumatoid Arthritis and C represents the pool of 20 controls from healthy subjects. Red arrows indicate the highly expressed proteins in patient's sera while green arrow indicates the expression of proteins with molecular weight less than 10 kDa.

The results also indicated the up-regulation of 50 kDa protein in 77 % (27/35) of patient's sera. Previously, this 50 kDa protein of unknown function and unidentified structure was isolated from human rheumatoid synovium, placenta and splenic tissues (Gunter and Josef, 2002). Autoantibodies against 50 kDa protein (Anti-Sa antibodies) have been observed in RA established patients (Hueber *et al*., 1999).

Moreover, many protein bands of 100 - 220kDa were also stained by more than 80 % of analyzed RA sera. But these heavy chain protein bands are not documented in literature to date against which antibodies had been reported.

Few sera of patients showed a stained band of less than 10 kDa molecular weight, interestingly no protein band of this molecular weight was stained by control sera.

Furthermore, autoantibodies are observed by using subsequent techniques such as Western blotting, ELISA or Dot blot etc followed by SDS-PAGE, whereas in our study we have used only SDS-PAGE for the profiling of differentially expressed proteins in RA patients.

CONCLUSION

It is concluded that the proteins expressed by RA patients can be further analyzed and detected by western blotting technique for the presence of autoantibodies.

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