DIFFERENTIAL EXPRESSION OF PROTEINS BY SDS-PAGE IN SERA OF PATIENTS SUFFERING FROM GASTRIC CANCER

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ABSTRACT

There are different proteins with different concentrations present in human serum, post-translational modifications or aberrant expression of these proteins is reported as signs which indicate the process of carcinogenesis in human body. The aim of the present study was to profile the differentially expressed serologic proteins using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. In this study fifty serum samples of gastric cancer (GC) patients and ten healthy donors were screened by SDS-PAGE to estimate the expression of proteins and to compare these proteins with the healthy donors used as control. The results showed that three different proteins were differentially expressed in GC patients while comparing with healthy donors used as control which can be further used as potential biomarkers to diagnose gastric cancer in its early stages.

Keywords: Gastric cancer, biomarkers and SDS-PAGE

INTRODUCTION

Gastric cancer (GC) is the second most common malignancy worldwide (Ferlay *et al*., 2015). In recent studies, GC accounts for 8% of the total cancer cases, 10% of the deaths for all cancers (Jemal *et al*., 2010) and 70% of the cases occurring in developing countries. These gastric cancer cases are due to *Helicobacter pylori* infection prevalence and nutritional routine. Changes in nutritional routine, improvement of can foods, reduction in smoking and significant decrease in *H. pylori* chronic infections lead to low GC incidence in developed countries (Chen *et al.*, 2007). The small percentage of gastric cancer have a familial component, with an autosomal pattern of inheritance and majority of stomach tumors are sporadic (Lastraioli *et al.*, 2012). Most of the incidences of gastric cancer are diagnosed at later stages when treatment and cure become almost impossible (Van Cutsem *et al.*, 2016). Therefore, it is important to search new non-invasive biomarkers for the early detection of GC (Sierzega *et al*., 2017).

Peptides present in human serum have been used as a biomarker for the identification of gastric cancer (Yang *et al*., 2012). Carbohydrate antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA), carbohydrate antigen 72-4 (CA 72-4) are the most common biomarkers available for GC (Uppal and Powell, 2013).

The survival rates for gastric cancer can be improved by early diagnosis and with proper treatment. Previous studies revealed several serum biomarkers for gastric cancer such as: CA 72-4, cancer antigen (CA) 19-9 and carcinoembryonic antigen, but sensitivity of these serum biomarkers is (20-30%) lower in diagnosis of gastric cancer as compared to other cancers (Fan and Xiong, 2011; Shimada *et al.*, 2014). It is essential to explore suitable biomarkers for the identification of GC (Pinheiro Ddo *et al*., 2014).

Proteomics based techniques are used to detect and measure proteins that can be used as biomarkers in tissues and body fluids in GC. Protein chip array, Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimensional electrophoresis (2-DE) are the proteomics based techniques used for the detection of GC (Uppal and Powell, 2013).

The aim of the present study was to predict the cellular proteins as diagnostic biomarkers recognized by autoantibodies in gastric cancer patients and to profile the differentially expressed proteins using Gel Electrophoresis.

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MATERIALS AND METHODS

Sample collection

Fifty serum samples were obtained from gastric cancer patients and 10 serum samples were taken from healthy subjects used as control from Bolan Medical Complex Hospital, Quetta (BMCH). Small vacutainer without anticoagulant were used to collect the serum samples. The blood samples of gastric cancer patients were centrifuged at 12000 rpm for 5-10 minutes to separate serum and then the separated serum samples were stored at -80°C for further use. According to the standard protocol, the estimation of protein concentration in collected serum samples was determined by using BCA protein kit (BOSTER) as per manufacturer instructions.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS –PAGE)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins on the bases of their molecular weight. The proteins were denatured by heating the samples and the unfolded proteins were coated with SDS detergent molecules which give negative charge to the proteins. For electrophoresis, Omni PAGE Mini (cleaver scientific Ltd.) system was used. In electrophoresis, two types of Polyacrylamide gels (resolving and stacking gel) were casted and serum samples were loaded after gel polymerization. The components used in polyacrylamide gels were as follows: Resolving gel (12% polyacrylamide), Tris 1.5M pH =8.8 (Molecular Biology Grade) 25%(v/v), SDS 10% (Bio-Rad Laboratories, Inc.) 1%(v/v), TEMED (Sigma Aldrich Co., Ltd.) 0.05%(v/v), ammonium persulfate10% (Prochem, Inc.-Rockford, IL) 0.5% (v/v) and in Stacking gel (4% polyacrylamide), Acrylamide (30%), Bis-acrylamide (0.8%) 13.3% (v/v), Tris 0.5M pH =6.8 (Molecular Biology Grade) 25%(v/v), SDS 10% (Bio-Rad Laboratories, Inc.) 1% (v/v), TEMED (Sigma Aldrich Co., Ltd.) 0.05% (v/v), Ammonium persulfate 10% (Prochem, Inc.-Rockford, IL) 0.5% (v/v). To visualize the protein bands, coomassie brilliant blue staining technique was used and images of stained gels were captured by using transilluminator apparatus (BIOBASE) (Smith, 1984).

RESULTS AND DISCUSSION

Different protein bands with weak and strong intensities were detected by SDS-PAGE in the presence of β-mercaptoethanol with little or no visible presence in normal as well as in patient's sera but may have the properties of having an electrophoresis profile, which are related to a clinical condition. The purpose of the study of the protein profiles obtained by SDS-PAGE was to predict biomarkers capable of differentiating the appearance of a pathological condition.

In this study fifty serum samples of gastric cancer patients and 10 healthy donors were screened by SDS-PAGE to observe the differential expression of proteins. The results shown that the bands of 34, 37, 45, 55 and 200 kDa were differentially stained in GC patient's sera comparing normal healthy sera (Fig.**1**). A 55 kDa thick band was seen in 74% (n=37) of GC patient's sera but this band was not observed in healthy donor's sera. Up-regulation of CALR (55-kDa protein, calreticulin) was observed in gastric cancer tissues by Ferlay *et al.* (2015) by western blotting analysis. The 55 kDa band we observed might correspond to the same protein CALR. In this study several proteins were separated by SDS-PAGE in the sera of patients diagnosed with GC as compared to the control group. A band of 200 kDa in 54% (n=27) of GC patient's sera was observed, this band might correspond to carcinoembryonic antigen (CEA). While comparing the sensitivity of CEA (40% and 100%), CA 72-4 (2.8% and 51.3%) and CA 19-9(5.6% and 68.2%) CEA has the highest sensitivity both in early and advanced stages of gastric cancer (Kim *et al*., 2011).

Carcinoembryonic antigen, a glycoprotein present on the cell surface, having a role in intracellular signaling, cell adhesion, and tumor progression (Oikawa *et al*., 1989). It is implicated as an oncogene by promoting cancer progression (Kochi *et al.,* 2000). CEA, as a tumor marker, firstly identified in 1965 (Gold and Freedman, 1965). This protein was functional for the early diagnosis of GC (Borch *et al.*, 1987). It is the most valuable serum protein marker used to diagnose gastric cancer at its early stage and to identify patients that have chances of developing GC. In previous studies, Kochi *et al.* (2000) proved that patients with high carcinoembryonic antigen (CEA) levels in their serum have high risk of having reappearance of the disease than the normal subjects. Kosugi *et al.* (2004) proposed that CEA levels in serum may be functional mostly in forecasting clinically in apparent metastasis and in GC, carcinoembryonic antigen(CEA) sensitivity was 30%, which denies Hwang *et al.* (2004) results (15.4% CEA sensitivity) stating that the sensitivity of CEA was not enough and beneficial in the detection of recurrence in GC patients.

A band of 80 kDa (stained with strong to moderate intensity) was observed in 52% (n=26) sera of GC patients in this study, this band resembles 80 kDa protein fragment, Soluble E-cadherin (sE-cad). In the results of this study

the 80 kDa protein band was also stained with a pool of control sera, the reason of this discrepancy was the limitations of the technique used. Since SDS-PAGE gives a matrix of separated proteins according to their molecular weight, while the Western blotting analysis provides confirmation of the separated proteins. We did not use Western blotting technique in our experiments that might predict Soluble E-cadherin (sE-cad) as a potential biomarker in prognosis, diagnosis and tumor reappearance of GC. Recently, the studies show that in GC, E-cadherin acts as a tumor suppressor gene and plays an initial and significant role in carcinogenesis (Liu and Chu, 2014).

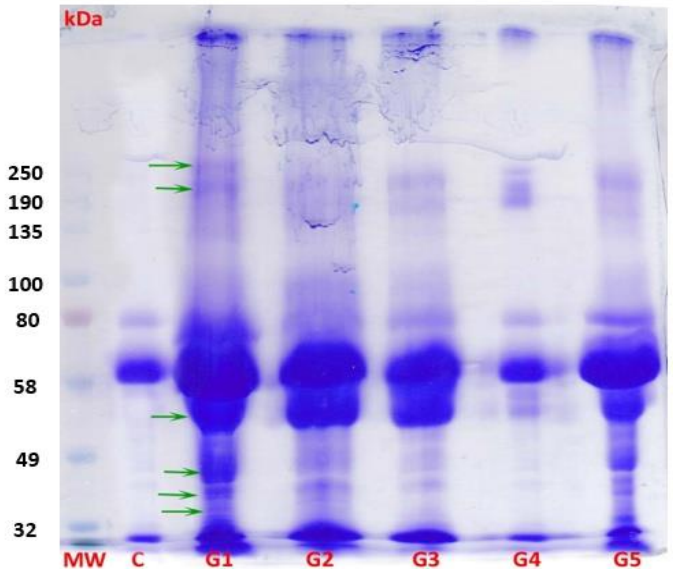

Fig.1.Representative CB staining gel illustrates staining pattern of differentially expressed serologic protein bands (depicted by green arrows) of gastric cancer patients comparing control sera. 'MW' indicates molecular weight, 'C' stands for pool of 10 control sera while 'G1-G5' show gastric cancer patient's sera.

A staining pattern of 34 kDa protein band was also observed in this study. Sera of 40% (n=20) GC patients were stained with this 34 kDa band. Lee *et al*. (2011) identified a protein spot of 34.3 kDa molecular weight as D-site binding protein by MALDI-TOF mass spectrometry from the cells of human GC patient's proteome and a metastatic OCUM- 2MLN cell line (Lee *et al*., 2011). In epidermal cells of mice, increased expression of D-site binding protein was observed to be associated with bFGF-regulated anchorage-independent growth response (Waters *et al*., 2009). Nevertheless, to establish the correlation of DBP expression with growth and/or metastasis of transformed cells, more evidences are still to be investigated. Another protein, aspartate aminotransferase (44.6 kDa molecular weight) was also identified by same group (Lee *et al*., 2011) in the same experiments performed on the human gastric cancer cell lines. A relatively identical band of 45 kDa was detected in our study by SDS-PAGE. Sera of 38% (n=19) GC patients were stained with the 45 kDa protein band with strong intensity.

Though, serum analysis is analytically difficult due to the high dynamic range of the proteins. So other techniques like western blotting and dot blotting are required for further analysis of high and low molecular weight serum proteins. In proteomics, advanced high-throughput techniques have become more successful to identify novel biomarkers for human cancers. There are several biomarkers used for the diagnosis of gastric cancer. Recently, the studies show that in GC, E-cadherin acts as a tumor suppressor gene and plays an initial and significant role in carcinogenesis (Liu and Chu, 2014).

Gene Ontology Analysis

Gene Ontology analysis was performed by using the PANTHER (pantherdb.org) Classification System which classifies proteins to facilitate high-throughput analysis (Fig.2). Proteins were classified according to their molecular functions, biological process, cellular component, protein pathways and protein class. The most common molecular function of proteins in GC patient's sera was 25% binding property and 75% catalytic activity. The cell component carries 28.6% cell part, 14.3% cell junction, 14.3% macromolecular complex, 14.3% membrane and 28.6% organelle. The major biological processes were 12.5% biological adhesion, 12.5% cellular component organization or biogenesis, 25% multicellular organismal process, 25% cellular process, 12.5% developmental process and 12.5%

metabolic process. PANTHER classification idetified 5 pathways with signaling mechanisms 20% Alzheimer diseaese-presenilin, 20% Asparagine and aspartate biosynthesis, 20% CCKR signaling map, 20% Cadherin signaling, 20% Wnt signaling and the protein classes were 50% calcium ion binding, 25% nucleic acid binding and 25% protein binding (Fig.2).

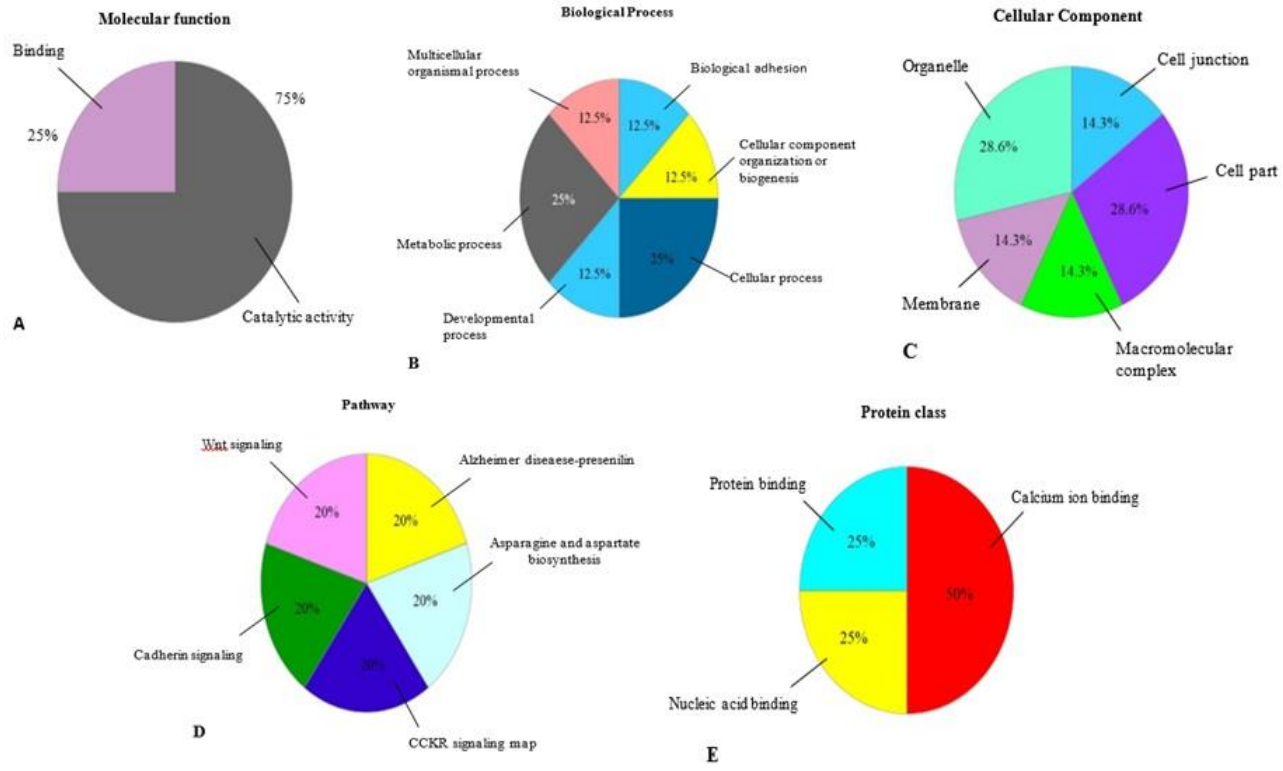

Fig. 2. Gene Ontology classification of proteins according to their: A. molecular functions, B. biological process, C. cellular component, D. protein pathways, E. protein class using PANTHER.

Conclusion

It is concluded that these proteins might play an important role in carcinogenesis during the transition phase and the autoantibodies against these proteins are generated long before clinical appearance of the disease. Hence, the autoantibodies to these antigens might be used as diagnostic biomarkers for early detection of gastric cancer. New biomarkers need to be identified and tested to determine their correlation with clinical parameters and predictions.

REFERENCES

Borch, K., H. Renvall, C. Lundin and B. Wahren (1987). Evaluation of gastric carcinoembryonic antigen analysis as an aid during screening for gastric neoplasia in atrophic gastritis. *Gut.*, 28(1): 26-32.

Chen, J., X.L. Bu, Q. Y. Wang, P. J. Hu and M.H. Chen (2007). Decreasing seroprevalence of Helicobacter pylori infection during 1993-2003 in Guangzhou, southern China. *Helicobacter*, 12(2): 164-169.

Fan, B. and B. Xiong (2011). Investigation of serum tumor markers in the diagnosis of gastric cancer. *Hepatogastroenterology*, 58(105): 239-245.

Ferlay, J., I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo and F. Bray (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*, 136(5): E359-386.

Gold, P. and S.O. Freedman (1965). Specific carcinoembryonic antigens of the human digestive system. *J Exp Med.*, 122(3): 467-481.

Hwang, G. I., C.H. Yoo, B. H. Sohn, J. H. Shin, Y. L. Park, H. D. Kim, Y.S. Kim, W.K. Han and W.K. Pae (2004). Predictive value of preoperative serum CEA, CA19-9 and CA125 levels for peritoneal metastasis in patients with gastric carcinoma. *Cancer Res. Treat.,* 36(3): 178-181.

Jemal, A., R. Siegel, J. Xu and E. Ward (2010). Cancer statistics, 2010. *CA Cancer J Clin.,* 60(5): 277-300.

Kim, D. H., S. J. Oh, C. A. Oh, M. G. Choi, J. H. Noh, T. S. Sohn and S. Kim (2011). The relationships between perioperative CEA, CA 19-9, and CA 72-4 and recurrence in gastric cancer patients after curative radical gastrectomy. *J Surg Oncol.,* 104(6): 585-591.

Kochi, M., M. Fujii, N. Kanamori, T. Kaiga, T. Kawakami, K. Aizaki and M. Yamagata (2000). Evaluation of serum CEA and CA19-9 levels as prognostic factors in patients with gastric cancer. *Gastric Cancer,* 3(4): 177-186.

Kosugi, S., T. Nishimaki, T. Kanda, S. Nakagawa, M. Ohashi and K. Hatakeyama (2004). Clinical significance of serum carcinoembryonic antigen, carbohydrate antigen 19-9, and squamous cell carcinoma antigen levels in esophageal cancer patients. *World J Surg.,* 28(7): 680-685.

Lastraioli, E., M. R. Romoli and A. Arcangeli (2012). Immunohistochemical biomarkers in gastric cancer research and management. *Int J Surg Oncol.,* 2012: 868645.

Lee, D. H., Y. Lee, J. Ryu, S. G. Park, S. Cho, J.J. Lee and B.C. Park (2011). Identification of proteins differentially expressed in gastric cancer cells with high metastatic potential for invasion to lymph nodes. *Mol Cells,* 31(6): 563-571.

Liu, X. and K.M. Chu (2014). E-cadherin and gastric cancer: cause, consequence, and applications. *Biomed Res Int.,* 2014: 637308.

Oikawa, S., C. Inuzuka, M. Kuroki, Y. Matsuoka, G. Kosaki and H. Nakazato (1989). Cell adhesion activity of non-specific cross-reacting antigen (NCA) and carcinoembryonic antigen (CEA) expressed on CHO cell surface: homophilic and heterophilic adhesion. *Biochem Biophys Res Commun.,* 164(1): 39-45.

Ordonez, C., R.A. Screaton, C. Ilantzis and C.P. Stanners (2000). Human carcinoembryonic antigen functions as a general inhibitor of anoikis. *Cancer Res.,* 60(13): 3419-3424.

Pinheiro Ddo, R., W.A. Ferreira, M. B. Barros, M. D. Araujo, S. Rodrigues-Antunes and N. Borges Bdo (2014). Perspectives on new biomarkers in gastric cancer: diagnostic and prognostic applications. *World J Gastroenterol.,* 20(33): 11574-11585.

Shimada, H., T. Noie, M. Ohashi, K. Oba and Y. Takahashi (2014). Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association. *Gastric Cancer,* 17(1): 26-33.

Sierzega, M., M. Kaczor, P. Kolodziejczyk, J. Kulig, M. Sanak and P. Richter (2017). Evaluation of serum microRNA biomarkers for gastric cancer based on blood and tissue pools profiling: the importance of miR-21 and miR-331. *Br J Cancer,* 117(2): 266-273.

Smith. B.J. (1984). SDS Polyacrylamide Gel Electrophoresis of proteins. *Methods Mol. Biol.,* 1: 41-55. doi: 10.1385/0-89603-062-8:41.

Uppal, D. S. and S.M. Powell (2013). Genetics/genomics/proteomics of gastric adenocarcinoma. *Gastroenterol Clin North Am.,* 42(2): 241-260.

Van Cutsem, E., X. Sagaert, B. Topal, K. Haustermans and H. Prenen (2016). Gastric cancer. *Lancet,* 388(10060): 2654-2664.

Waters, K. M., R. Tan, L.K. Opresko, R. D. Quesenberry, S. Bandyopadhyay, W.B. Chrisler and T.J. Weber (2009). Cellular dichotomy between anchorage-independent growth responses to bFGF and TPA reflects molecular switch in commitment to carcinogenesis. *Mol Carcinog.,* 48(11): 1059-1069.

Yang, J., Y.C. Song, C. X. Dang, T. S. Song, Z. G. Liu, Y. M. Guo, Z. F. Li and C. Huang (2012). Serum peptidome profiling in patients with gastric cancer. *Clin Exp Med,* 12(2): 79-87.

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