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Significance of Complement factor and Immunoglobulin in *Helicobacter pylori* induced Gastric Cancer

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Abstract:

Background: Gastric cancer (GC) still found to be the predominant cancer in Asia.There were very few reports which were focused on population specific biomarker studies with respect to *Helicobacter pylori* (*H. pylori)* induced gastric cancer in India. Hence, we aimed to perform this biomarker study involving *H. pylori* positive and negative healthy serum samples in comparison with *H. pylori* positive gastric cancer samples.

Methods: We have adapted Proteome based approaches to identify serum biomarkers in our Indian population samples. For proteins separation and identification, 2D gel electrophoresis with tandem mass spectrometry approach was performed and thirty differentially regulated protein spots were analyzed.

Results: In comparison of *H. pylori* negative, *H. pylori* positive healthy serum samples with *H. pylori* positive gastric cancer serum samples, four proteins including Prohibitin 2, Serum albumin, Apolipoprotein E and Complement factor B were downregulated and eleven proteins including haptoglobin and its isoform, serotransferrin, Immunoglobulins, Apolipoprotein A1, complement factor C3 proteins were upregulated.

Conclusion:A panel of proteins, rather than one single protein is a more accurate candidate for a diagnostic marker. In this study, we found, along with other proteins, previously unreported protein- IGKC to be involved in the pathophysiology of gastric cancer.

Keywords: Serum biomarkers; Gastric Cancer; Proteomics; 2D gel electrophoresis; Mass spectrometry; Cancer Biomarkers

1. Introduction

Globally, among predominant human cancers, Gastric cancer still found to be a major issue for mankind and known to have higher incidence in Asia [1]. Mostly, gastric cancer occurs without showing any major symptoms in their initial stage and it is very difficult to identify them[2].The only best treatment option that was successful is performing surgery and it was found to be best compared to major developments in chemotherapy. However, when comes toprognosis, it always getting worsen and the survival rate is also very minimal with respect to late stage of gastric cancer. So obviously we need to catch hold of this gastric cancer in a very early stage by which eventually we shall have a better outcome with respect to treatment strategies.

The most commonly and routinely used diagnosis method with respect to gastric cancer is endoscopy and biopsy. But to execute all these methods, highly trained professionals are required. It is highly costlier and as well as invasive which might causes the patients uncomfortable. Even after seeing multiple levels of advancements in identifying the molecular biomarkers which can diagnose gastric cancer in early stage itself [3], the complete usefulness of diagnostic markers for gastric cancer still not achieved. Certain established markers have been used for the early diagnosis, to determine the prognosis, to monitor the disease recurrence[4,5].But most often used serum biomarkers are of less use as they don't

possess high accuracy in detecting the gastric cancer[6].Hence, there should be additional promising markers to identify the gastric cancer in very early stage. In any biological system, the proteins are getting altered during metabolic changes happening in the tumorigenesis process.Emerging high throughput techniques including tandem mass spectrometryare helpful in screening large number of proteins. Very limited reports were available with respect to identification of biomarkers using Indian population. Keeping these points in mind, we framed our objectives to identify novel biomarkers using serum samples of Indian population.

2. Materials and Methods

2.1.Ethical approval, recruitment of study subjects and sample collection

The proposed study was approved by the Institutional Ethics Committee of Madras Medical College (MMC), Chennai and allotted with IEC No. 31082015. The study subjects were of South Indian Tamil origin and comprised of 40 subjects, of which tensamples (n =10) were included as healthy*H.pylori*negative control and twentysamples (n=20) under the *H.pylori*positive gastric cancer and*H. pylori*positive healthy cases (n=10). The patients with the complaint of persistent pain in the abdomen, satiety, nausea, and dysphagia were subjected to gastrointestinal endoscopy at Medical Gastroenterology unit, Rajiv Gandhi Government General Hospital (MMC-RGGGH), Chennai, India.The endoscopic observation and on further evaluation revealed that gastric cancercases (n=20) were presented with bleeding from growth along with gastric outlet obstruction with the involvement of serosal and lymph. The gastric biopsy specimen obtained from pylori antrum was tested for *H. pylori*infectionusing Rapid Urease Test (RUT kit, Gastro cure systems, Kolkata). The samples tested positive by rapid urease test will be included as *H. pylori* positive cases. The study subjects were obtained with written informed consent and their blood samples were collected during the period August 2019 to December 2019. Serum from their whole blood samples was collected and stored at -80˚C until further processing.

2.2.Two-dimensional Gel Electrophoresis (2DGE) and staining technique

The samples were thawed and pool of samples was prepared. This pooling is done to understand the information for a set of same category of patients. For making the pools, the proteins estimation was performed for all the samples. Equal concentration of proteins i.e 100µg from each sample was mixed together in a ratio of five samples in each pool. Totally four pools were made in the same way. Total protein taken for two dimensional electrophoresis (2DE) was 100µg for each gel from each pool. The protein was added to 350 µL optimized 2D rehydration buffer [7 M urea, 0.5% ampholytes, 4% CHAPS, 2 M thiourea, 50 mMdithiothreitol, and 0.004% of bromophenol blue]with slight modifications[7]. The IPG strips were placed on this buffer and kept for 12-16 hrs incubation. After that the IPG strips were focussed in IPGphor machine and voltage was applied to each 82 kV at 20˚C. The following parameters were used: 500V step-n-hold for 1h, 1 kV gradient for 1 h, 8kV gradient for 3 h and 8 kV step-n-hold for 8h. After focusing, the strips were stored at -70 ˚C overnight. Further these strips were treated with equilibration buffers and then 2DE was run

on SE 600 (GE Healthcare, Uppsala, Sweden)apparatus[8, 9]. To control the experimental variation, all the samples were processed together.Further these gels were stained with CBB G 250 stain. Briefly, the gels were fixed with 40% methanol and 10% acetic acid for 1 hr. Water wash for 10 minutes was given thrice after fixing. Overnight CBB G 250 stain was treated after water wash. After staining, the stains were removed using water[10].

2.2.1. In-gel Trypsindigestion

In-gel trypsin digestion was performed as detailed earlier [10]. Briefly, spots were washed with water (10 min). The spots were destained with 25 mM ammonium bicarbonate (AmBic) in 50% acetonitrile (ACN) multiple times, till the color of the gelgets removed completely. Further the spots were treated with 100% ACN for 15 min, then vaccum dried for 30 min. Then 500ng trypsin dissolved in 5 µL 100 mMAmBic(in 10% ACN) was added for 30 min and kept in ice. To that, 30 µL of 40 mMAmBic(in 10% ACN) were added to the spots and kept for 12 hrs incubation at room temperature. Finally, 35 µL of 0.1% trifluoroacetic acid (TFA; in 50% ACN) were added to thespots and incubated for10 min. Both supernatants were mixed andvacuum dried. The dried peptides were suspended in 8 µL of 0.1% TFA (in 5% ACN).

2.3. Matrix-assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) Spectrometry of Tryptic Digests

The samples were subjected toMSanalysis as detailed earlier [7]. The α-cyano-4-hydroxycinnamic acid (CHCA) matrix (2 mg/mL, prepared in 70% ACN and 0.03% TFA) was applied in this study. Sandwich method was adopted for the peptide sampleapplication on stainless steel MALDI target plate. The CHCA matrix (0.5 µL) was layered first followed by sample of same volume on the plate and allowed to dry and followed by 0.5 µL of the matrix layered on top of the sample. The reflector mode was adopted to acquire the peptide mass spectrum by MS analysis. An acceleration voltage of 20 kV was fixed to pulsed extraction and the calibration standards used were Bradykinin (757.39 Da), P14R (1533.85 Da), angiotensin II (1046.54 Da), and adrenocorticotropic hormone fragment (2465.19 Da). Search parameters in the database were set as detailed earlier [10].Databases adapted for the search wasNCBInr, MSDB, and SWISS-PROT.

2.4. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Analysis

With slight minor modification, the In-gel tryptic digestion was carried out as described earlier [8]. The samples were injected to a nano-liquid chromatography system (UltiMate^{TM} 3000, Dionex^{TM}, Hong Kong)coupled with Electrospray Ionization-Quadrupole-Time of Flight (micrOTOF-Q, BrukerDaltonics). The MASCOT software v2.2 (Matrix Science, London, UK) was helpful in performing the post spectrum analysis and database search. MS/MS ion tolerance value of 0.1Da and peptide tolerance of 0.2Da was included for search parameters while the other parameters were performed as earlier [10].

2.5 Spot Quantitation and Statistical Analysis

Image analysis was performed using ImageMaster 2D Platinum 7.0 (IMP7; GE Healthcare, Hong Kong, China). The protein spots were matched for spot intensity and the statistical analysis used for analyzing the spot comparison was the analysis of variance (ANOVA).

2.6. Gene Ontology forProteins

Protein Analysis THrough Evolutionary Relationships (PANTHER)database [11] was used for gene ontology analysis, where the gene names of differentially expressed proteins were given as an input. PANTHER helps in the classification of a large curate biological database of gene or protein families and their functionally related subfamilies. This was done based on molecular function, biological process, and cellular localization categories. For the construction of protein networks[12] Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org) has been used. Based on genomic context, experiments with high throughput, previous knowledge, and conserved co-expression, the interaction can beeither direct (physical), indirect (functional), or both. Integration maps were generated based onthese quantitatively derived interaction data.

3. Results and Discussion

3.1.2D Spot Analysis with IMP7

The protein spots from Coomassie stained gels were selected and compared using the ImageMaster 2D platinum version 7.0 (IMP 7) software (GE healthcare) and quantified (Figure 1). A total of 43 spots could be detected in eleven gels. Among the 43 spots, the differencecould be measured for 30 spots. The differentially expressed spots were statistically analysed to identify the significantly deregulated spots. To minimize the error in calculating the intensity difference of spots between the gels, the upregulation and downregulation was analyzed by calculating the ratio of percent intensity across all biological triplicate gels.

In comparison of HP negative healthy, HP positive healthy and HP positive gastric cancer, seventeen spots showed differential expression which were statistically significant and details of class analysis table using IMP7 was represented in Table 1. Among them, twelve spots were upregulated and five spots were downregulated in gastric cancer samples. The percentage of relative intensity of all the thirty protein spots was depicted in Figure 2.

3.2. Differential Expression of Serum Proteins

All the fifteen differentially expressed spots were subjected to mass spectrometry analysis through MALDI-TOF or LC-MS/MS analysis and the details were depicted in Table 2. It describes the identified protein name, accession number, mass spec score, sequence coverage percentage, gene ID, number of unique peptides, amino acid number, molecular weight and pI. The list of proteins includes haptoglobin and its isoforms, prohibitin 2, serotransferrin,

serum albumin, Immunoglobulin and compliment, apolipoprotein A1 and E and C reactive protein.

In comparison of *H. pylori* negative, *H. pylori* positive healthy serum samples with *H pylori* positive gastric cancer serum samples, four proteins including Prohibitin 2, Serum albumin, Apolipoprotein E and Complement factor B were downregulated and eleven proteins including haptoglobin and its isoform, serotransferrin, Immunoglobulins, Apolipoprotein A1, complement factor C3 proteins were upregulated.

In comparison of *H pylori* negative healthy serum samples with *H pylori* positive gastric cancer serum samples, three proteins including Prohibitin 2, Serum albumin, Apolipoprotein E were downregulated and twelve proteins including haptoglobin and its isoform, serotransferrin, Immunoglobulins, Apolipoprotein A1, complement proteins were upregulated.

In comparison of *H pylori* positive healthy serum samples with *H pylori* positive gastric cancer serum samples, four proteins including Prohibitin 2, Serum albumin, Apolipoprotein E and Complement factor B were downregulated and eleven proteins including haptoglobin and its isoform, serotransferrin, Immunoglobulins, Apolipoprotein A1, complement factor C3 proteins were upregulated.

3.3. Functional Classification of Identified Proteins and Biological Network Analysis

Protein AnalysisTHrough Evolutionary Relationships (PANTHER) [11] analysis was performed to gain better understanding on the functions of all the differentially expressed proteins identified through mass spectrometry. All differentially expressed proteins were categorized into three groups based on their molecular function, biological process and cellular localization. In the molecular function category, 31.30% of the proteins were involved in Binding activity, 18.80% of proteins involved in Catalytic activity, 6.30% of the proteins involved in Molecular Transducer Activity, 6.30% of the proteins involved in Structural Molecule Activity and 6.30% of the proteins involved in molecular function regulator. In the biological process category, 31.30% involved in cellular component organization or biogenesis, 50% of the proteins involved in cellular process, 12.50% of the proteins involved in multi organism process, 31.30% of the proteins involved in localization, 37.50% of the proteins involved in biological regulation, 31.30% of the proteins involved in respond to stimulus, 18.80% of the proteins involved in signaling, 6.30% involved in developmental process and multicellular organismal process, 31.30% involved in metabolic process and 12.50% involved in immune system process.

Among the 15 differentially expressed proteins, with respect to cellular localization category, 25% showing to be membrane, 25% showing to be membrane part, 12.50% showing to be organelle part, 37.50% showing to be extracellular region part, 25% showing to be protein containing complex, 6.30% showing to be supramolecular complex, 37.50% showing to be extracellular region, 37.50% showing to be cell, 37.50% showing to be cell part and 12.50% showing to be organelle. And, with respect to Protein class 6.30% of the

proteins involved in transmembrane signal receptor, 12.50% involved in transfer/ carrier protein, 18.80% involved in defense/ immunity protein, 6.30% involved in cytoskeletal protein and 12.50% of the proteins involved in protein modifying enzyme.

In this study, the serum proteomic profiles of gastric cancer patients were analyzed using 2DE and were validated through mass spectrometry. Several differentially expressed proteins, including novel which were previously unreported ones, were identified with statistically significant variations in expression levels, as compared to control samples. Among the identified targets complement factor and immunoglobulin expression pattern were not much explored before with respect to *H.pylori* induced gastric cancer. Hence we did more analysis with these two set of proteins and discussed in this paper.

3.3.1. Importance of complement factor and Immunoglobulin

The comparative serum proteomic profiling reveals differential protein expression of novel targets in *H.pylori* induced gastric cancer from Indian population.For all clinical diagnostic procedures, serum sample is very suitable as it is readily available and procuring it is a non invasive procedure. As serum is easily approachable sample with fewer complications, it is used for detecting effective biomarkers.

There might be multiple research studies to discuss about the process of complement activation and accumulation in many type of tumor tissue but the role and significance as such is not yet completely understood. Studies found that the complement fails to kill the cancer cells which might be due to its resistance against complement attack[13]. The role of complement is not been studied in detail in the aspect of malignant neoplasms, but the application mAbs to treat cancer has encouraged scientist to perform more studies in complement activation in combination with antibody immunotherapy [14-16]. Multiple research studies have described that these complement and its components are been accumulated in different type of cancer tissues [17-19]. Recent research aspects are beingchallenging this dogma now, as most of the complement and its components were associated with cancer hallmarks but showing an opposing effect[20].

The role of complement is to provide the defensive role against the entire microorganism with a higher efficiency towards gram-negative organisms [21]. Often the levels of immunoglobulin will be decreased and in thosepatients it is a warning signal that they might get infection easily[22,23].In contrast, there was observed a significant upregulation of these immunoglobulins specifically Ig A and Complements in cancer patients [24] who are undergoing surgery and in those who havechronic infections [21]. In oesophageal cancer, these levels were elevated in surgery patients with chronic infection [25]. Multiple studies have found that the increase of levels and its relation to age and sex[26,27]tumors[28,29] and protein-calorie malnutrition (PCM) [30]. Authors reported the increase of IgA in oesophageal cancer [31] and in malnutrition [32]. As not much studies focused in elucidating the functional role involved in these scenarios, it is not well clarified about the process and mechanism involved.

In other hand,complement and its components are not elevated at all in patients with PCM [30]. With respect to tumor patients, the levels of complement and its components are differentially expressed and these details were observed in serum samples[29] and these complement levels were also noted to be differentially regulated in gastric cancer patients of different population [33] and in surgery patients [34].

Figure 3A, 3B & 3C represents functional characterization including Biological process, Molecular function & Cellular function of complement C2, complement factor B and immunoglobulins and Figure 4 represents the Protein protein interaction analysis for complement C2, complement factor B and immunoglobulins using STRING analysis.

4. Conclusion

In our study, we found differential regulation of complement factor and immunoglobulins in*H.pylori* induced gastric cancer serum samples. These results suggests that these promising markers may play a prognostic role in identifying the *H.pylori* induced gastric cancer in an very early stage in Indian population.

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Conflict of Interest

All the authors have no potential conflict of Interest to disclose.

References

1. Hartgrink, H. H.; Jansen, E. P. M.; van Grieken, N. C. T.; van de Velde, C. J. H. Gastric Cancer. *Lancet***2009**, *374* (9688), 477–490. https://doi.org/10.1016/S0140-6736(09)60617-6
2. Hunt, R. H.; Camilleri, M.; Crowe, S. E.; El-Omar, E. M.; Fox, J. G.; Kuipers, E. J.; Malfertheiner, P.; McColl, K. E. L.; Pritchard, D. M.; Rugge, M.; Sonnenberg, A.; Sugano, K.; Tack, J. The Stomach in Health and Disease. *Gut***2015**, *64* (10), 1650–1668. https://doi.org/10.1136/gutjnl-2014-307595.
3. González, C. A.; Agudo, A. Carcinogenesis, Prevention and Early Detection of Gastric Cancer: Where We Are and Where We Should Go. *Int J Cancer***2012**, *130* (4), 745–753. https://doi.org/10.1002/ijc.26430.
4. Emoto, S.; Ishigami, H.; Yamashita, H.; Yamaguchi, H.; Kaisaki, S.; Kitayama, J. Clinical Significance of CA125 and CA72-4 in Gastric Cancer with Peritoneal

Dissemination. *Gastric Cancer***2012**, *15* (2), 154–161. https://doi.org/10.1007/s10120-011-0091-8.

5. Li, Y.; Yang, Y.; Lu, M.; Shen, L. Predictive Value of Serum CEA, CA19-9 and CA72.4 in Early Diagnosis of Recurrence after Radical Resection of Gastric Cancer. *Hepatogastroenterology***2011**, *58* (112), 2166–2170. https://doi.org/10.5754/hge11753.
6. Shimada, H.; Noie, T.; Ohashi, M.; Oba, K.; Takahashi, Y. 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***2014**, *17* (1), 26–33. https://doi.org/10.1007/s10120-013-0259-5.
7. Ananthi, S.; Lakshmi, C. N. P.; Atmika, P.; Anbarasu, K.; Mahalingam, S. Global Quantitative Proteomics Reveal Deregulation of Cytoskeletal and Apoptotic Signalling Proteins in Oral Tongue Squamous Cell Carcinoma. *Scientific Reports***2018**, *8* (1), 1–13. https://doi.org/10.1038/s41598-018-19937-3.
8. Ananthi, S.; Prajna, N. V.; Lalitha, P.; Valarnila, M.; Dharmalingam, K. Pathogen Induced Changes in the Protein Profile of Human Tears from Fusarium Keratitis Patients. *PLOS ONE***2013**, *8* (1), e53018. https://doi.org/10.1371/journal.pone.0053018.
9. Ananthi, S.; Santhosh, R. S.; Nila, M. V.; Prajna, N. V.; Lalitha, P.; Dharmalingam, K. Comparative Proteomics of Human Male and Female Tears by Two-Dimensional Electrophoresis. *Experimental Eye Research***2011**, *92* (6), 454–463. https://doi.org/10.1016/j.exer.2011.03.002.
10. Ananthi, S.; Chitra, T.; Bini, R.; Prajna, N. V.; Lalitha, P.; Dharmalingam, K. Comparative Analysis of the Tear Protein Profile in Mycotic Keratitis Patients. *Mol Vis***2008**, *14*, 500–507.
11. Mi, H.; Huang, X.; Muruganujan, A.; Tang, H.; Mills, C.; Kang, D.; Thomas, P. D. PANTHER Version 11: Expanded Annotation Data from Gene Ontology and Reactome Pathways, and Data Analysis Tool Enhancements. *Nucleic Acids Res.***2017**, *45* (D1), D183–D189. https://doi.org/10.1093/nar/gkw1138.
12. Szklarczyk, D.; Franceschini, A.; Wyder, S.; Forslund, K.; Heller, D.; Huerta-Cepas, J.; Simonovic, M.; Roth, A.; Santos, A.; Tsafou, K. P.; Kuhn, M.; Bork, P.; Jensen, L. J.; von Mering, C. STRING V10: Protein-Protein Interaction Networks, Integrated over the Tree of Life. *Nucleic Acids Res.***2015**, *43* (Database issue), D447-452. https://doi.org/10.1093/nar/gku1003.
13. Fishelson, Z.; Donin, N.; Zell, S.; Schultz, S.; Kirschfink, M. Obstacles to Cancer Immunotherapy: Expression of Membrane Complement Regulatory Proteins (MCRPs) in Tumors. *Mol Immunol***2003**, *40* (2–4), 109–123. https://doi.org/10.1016/s0161-5890(03)00112-3.
14. Gelderman, K. A.; Kuppen, P. J. K.; Bruin, W.; Fleuren, G. J.; Gorter, A. Enhancement of the Complement Activating Capacity of 17-1A MAb to Overcome the Effect of Membrane-Bound Complement Regulatory Proteins on Colorectal Carcinoma. *European Journal of Immunology***2002**, *32* (1), 128–135. https://doi.org/10.1002/1521-4141(200201)32:1<128::AID-IMMU128>3.0.CO;2-P.

15. Sier, C. F. M.; Gelderman, K. A.; Prins, F. A.; Gorter, A. Beta-Glucan Enhanced Killing of Renal Cell Carcinoma Micrometastases by Monoclonal Antibody G250 Directed Complement Activation. *Int J Cancer***2004**, *109* (6), 900–908. https://doi.org/10.1002/ijc.20029.
16. Allendorf, D. J.; Yan, J.; Ross, G. D.; Hansen, R. D.; Baran, J. T.; Subbarao, K.; Wang, L.; Haribabu, B. C5a-Mediated Leukotriene B4-Amplified Neutrophil Chemotaxis Is Essential in Tumor Immunotherapy Facilitated by Anti-Tumor Monoclonal Antibody and β-Glucan. *The Journal of Immunology***2005**, *174* (11), 7050–7056. https://doi.org/10.4049/jimmunol.174.11.7050.
17. Lucas, S. D.; Karlsson-Parra, A.; Nilsson, B.; Grimelius, L.; Akerström, G.; Rastad, J.; Juhlin, C. Tumor-Specific Deposition of Immunoglobulin G and Complement in Papillary Thyroid Carcinoma. *Hum Pathol***1996**, *27* (12), 1329–1335. https://doi.org/10.1016/s0046-8177(96)90346-9.
18. Blok, V. T.; Daha, M. R.; Tijsma, O. M.; Weissglas, M. G.; van den Broek, L. J.; Gorter, A. A Possible Role of CD46 for the Protection in Vivo of Human Renal Tumor Cells from Complement-Mediated Damage. *Lab Invest***2000**, *80* (3), 335–344. https://doi.org/10.1038/labinvest.3780038.
19. Niculescu, F.; Rus, H. G.; Retegan, M.; Vlaicu, R. Persistent Complement Activation on Tumor Cells in Breast Cancer. *Am J Pathol***1992**, *140* (5), 1039–1043.
20. Markiewski, M. M.; Lambris, J. D. Is Complement Good or Bad for Cancer Patients? A New Perspective on an Old Dilemma. *Trends Immunol***2009**, *30* (6), 286–292. https://doi.org/10.1016/j.it.2009.04.002.
21. Grieco, M. H. *Infections in the Abnormal Host*; Yorke Medical Books, 1980.
22. Bjornson, A. B.; Altemeier, W. A.; Bjornson, H. S. Changes in Humoral Components of Host Defense Following Burn Trauma. *Ann. Surg.***1977**, *186* (1), 88–96. https://doi.org/10.1097/00000658-197707000-00012.
23. Nohr, C. W.; Latter, D. A.; Meakins, J. L.; Christou, N. V. In Vivo and in Vitro Humoral Immunity in Surgical Patients: Antibody Response to Pneumococcal Polysaccharide. *Surgery***1986**, *100* (2), 229–238.
24. Hughes, N. R. Serum Concentrations of γG, γA, and γM Immunoglobulins in Patients With Carcinoma, Melanoma, and Sarcoma*JNCI: Journal of the National Cancer Institute***1971**, No. 46, 1015–1028. https://doi.org/10.1093/jnci/46.5.1015.
25. Saito, T.; Kuwahara, A.; Shimoda, K.; Kinoshita, T.; Shigemitsu, Y.; Miyahara, M.; Kobayashi, M. Enhanced Immunoglobulin Levels Correlate with Infectious Complications after Surgery in Esophageal Cancer. *J Surg Oncol***1991**, *46* (1), 3–8. https://doi.org/10.1002/jso.2930460103.
26. Kishimoto, S.; Tomino, S.; Inomata, K.; Kotegawa, S.; Saito, T.; Kuroki, M.; Mitsuya, H.; Hisamitsu, S. Age-Related Changes in the Subsets and Functions of Human T Lymphocytes. *The Journal of Immunology***1978**, *121* (5), 1773–1780.
27. Nagaki, K.; Hiramatsu, S.; Inai, S.; Sasaki, A. The Effect of Aging on Complement Activity (CH50) and Complement Protein Levels. *J Clin Lab Immunol***1980**, *3* (1), 45–50.
28. Brown, A. M.; Lally, E. T.; Frankel, A.; Harwick, R.; Davis, L. W.; Rominger, C. J. The Association of the IgA Levels of Serum and Whole Saliva with the Progression

of Oral Cancer. *Cancer***1975**, *35* (4), 1154–1162. https://doi.org/10.1002/1097-0142(197504)35:4<1154::AID-CNCR2820350421>3.0.CO;2-D.

29. Nishioka, K.; Kawamura, K.; Hirayama, T.; Kawashima, T.; Shimada, K. The Complement System in Tumor Immunity: Significance of Elevated Levels of Complement in Tumor Bearing Hosts. *Ann. N. Y. Acad. Sci.***1976**, *276*, 303–315. https://doi.org/10.1111/j.1749-6632.1976.tb41656.x.
30. Gross, R. L.; Newberne, P. M. Role of Nutrition in Immunologic Function. *Physiol. Rev*.**1980**, *60* (1), 188–302. https://doi.org/10.1152/physrev.1980.60.1.188.
31. Haffejee, A. A.; Angorn, I. B. Nutritional Status and the Nonspecific Cellular and Humoral Immune Response in Esophageal Carcinoma. *Ann Surg***1979**, *189* (4), 475–479.
32. Advani, S. H.; Kutty, P. M.; Gopal, R.; Swaroop, S.; Nair, C. N.; Dinshaw, K. A.; Damle, S. R.; Nadkarni, J. S.; Akolkar, P. N.; Gothaskar, B. P.; Vyas, J. J.; Desai, P. B. Immunity in Esophageal Carcinoma. *Journal of Surgical Oncology***1983**, *24* (4), 268–273. https://doi.org/10.1002/jso.2930240406.
33. Saito, T.; Kuwahara, A.; Shigemitsu, Y.; Kinoshita, T.; Shimoda, K.; Miyahara, M.; Kobayashi, M.; Shimaoka, A. Factors Related to Malnutrition in Patients with Esophageal Cancer. *Nutrition***1991**, *7* (2), 117–121.
34. Meakins, J. L.; Pietsch, J. B.; Christou, N. V.; Maclean, L. D. Predicting Surgical Infection before the Operation. *World J. Surg.***1980**, *4* (4), 439–449. https://doi.org/10.1007/BF02393171.

Table 1. Class analysis table with statistical analysis

Match ID	Max	Fold	Match Count	HP-ve Healthy (n=10)	HP+ve GC(n=20)	HP +ve Healthy(n=10)	ANOVA
0	3.49863	3.32521	3	1.05215	3.49863	1.93551	0.0212726
1	1.46088	1.57722	3	1.46088	0.926232	1.1565	0.228674
2	1.10801	2.3605	3	1.10801	0.469396	1.01703	0.156336
3	1.41238	1.10663	2	1.2763	-	1.41238	0.0262642
4	2.23591	6.63762	3	0.336853	2.23591	0.349093	0.0442058
5	2.72402	7.6709	3	1.27446	0.35511	2.72402	3.79336E-06
6	4.53347	8.08876	3	0.560466	4.53347	0.836982	1.92538E-05
7	1.10034	4.54095	3	0.242316	1.10034	0.407992	0.0414606
8	0.968984	2.60626	3	0.968984	0.371791	0.809388	0.0907822
9	4.26505	1.67522	3	4.26505	2.54597	2.99208	0.338255
10	2.51249	1.82252	3	2.51249	1.37858	1.77911	0.00787574
11	0.690932	2.64642	3	0.293242	0.261082	0.690932	0.0743308
12	2.36011	5.96173	3	0.395876	2.36011	0.424334	0.000903918
13	1.67871	1.47001	3	1.50049	1.14197	1.67871	0.357987
14	0.588489	9.69773	3	0.461059	0.0606832	0.588489	0.126718
15	2.81565	2.40796	3	2.15943	1.16931	2.81565	0.151085

16	2.53824	2.12763	3	1.70645	2.53824	1.19299	0.0266513
17	0.9368	1.31579	2	-	0.711966	0.9368	0.0276258
18	3.56338	2.06474	2	-	3.56338	1.72582	0.0157876
19	2.41992	7.21461	3	0.335419	2.41992	2.01105	0.0499422
20	2.26119	1.00737	2	-	2.26119	2.24464	0.0512368
21	2.82209	3.37114	3	0.837133	2.82209	1.65465	0.0483154
22	1.19714	1.66969	2	-	0.716985	1.19714	0.0267235
23	3.88695	2.86735	3	3.88695	1.35559	1.63845	0.256106
24	4.08148	2.07969	2	-	4.08148	1.96254	0.0319435
25	5.32286	2.60864	3	2.04047	5.32286	3.24313	0.0555995
26	2.73573	3.08649	3	0.886355	2.73573	2.50776	0.531596
27	6.31757	1.39484	3	6.31757	5.40528	4.52926	0.670094
28	2.69175	1.48669	3	1.81056	2.69175	2.28008	0.707104
29	13.4643	1.33249	3	11.2918	10.1046	13.4643	0.594367

HP-*Helicobacter pylori*

Table 2. Protein Identification Table

Match ID	**Accession No**	**Gene ID**	**Description**	**Score**	**Coverage %**	**# Proteins**	**# Unique Peptides**	**# Peptides**	**# PSMs**	**# AAs**	**MW (kDa)**	**calc. pI**
0	P00738	HP	Haptoglobin	53.78	33.99	8	7	19	27	406	45.2	6.58
3	J3KPX7	PHB2	Prohibitin-2	56.5	47.16	10	11	11	16	299	33.4	9.8
4	P02787	TF	Serotransferrin	570.38	44.99	10	30	43	259	698	77	7.12
5	H0YA55	ALB	Serum albumin (Fragment)	24.17	15.86	12	2	7	14	454	51.5	6.95
6	P01834	IGKC	Ig kappa chain C region	19.63	49.06	10	3	3	5	106	11.6	5.87
7	P02647	APOA1	Apolipoprotein A-I	128.66	80.9	4	19	36	73	267	30.8	5.76
10	P02649	APOE	Apolipoprotein E	46.55	64.35	10	10	20	30	317	36.1	5.73
12	P02741	CRP	C-reactive protein	33.51	25.45	3	6	8	20	224	25	5.63
16	P06681	C2	Complement C2	133.20	49.69	7	18	33	49	752	83.27	7.23
17	P00751	CFB	Complement	100.	32.65	13	11	23	48	76	85.5	6.6

			factor B	62						4	3	7
18	J3QLC9	HP	Haptoglobin (Fragment)	226.06	16.44	12	7	10	102	365	40.8	5.82
20	J3QLC9	HP	Haptoglobin (Fragment)	246.67	19.45	12	7	11	105	365	40.8	5.82
21	P00739	HPR	Haptoglobin-related protein	42.79	22.7	8	1	4	21	348	39	7.09
24	O75015	FCGR3B	Low affinity immunoglobulin gamma Fc region receptor III-B	82.74	60.48	4	6	11	33	233	26.22	6.22
25	B9A064	IGLL5	Immunoglobulin lambda-like polypeptide 5	20.29	56.07	13	2	9	19	214	23.05	8.84

The contents in table was provided with Match ID, accession number, gene name description of protein name, score,coverage %, number of peptides covered, peptide spectrum match (#PSM), amino acid number (#AAs), molecular weight (MW) , calculated isoelectric point (Calc.pI)

Figure 1. IMP7 gel analysis for the serum samples pools (A) HP negative healthy, (B) HP positive gastric cancer, (C) HP positive healthy separated based on their class category.

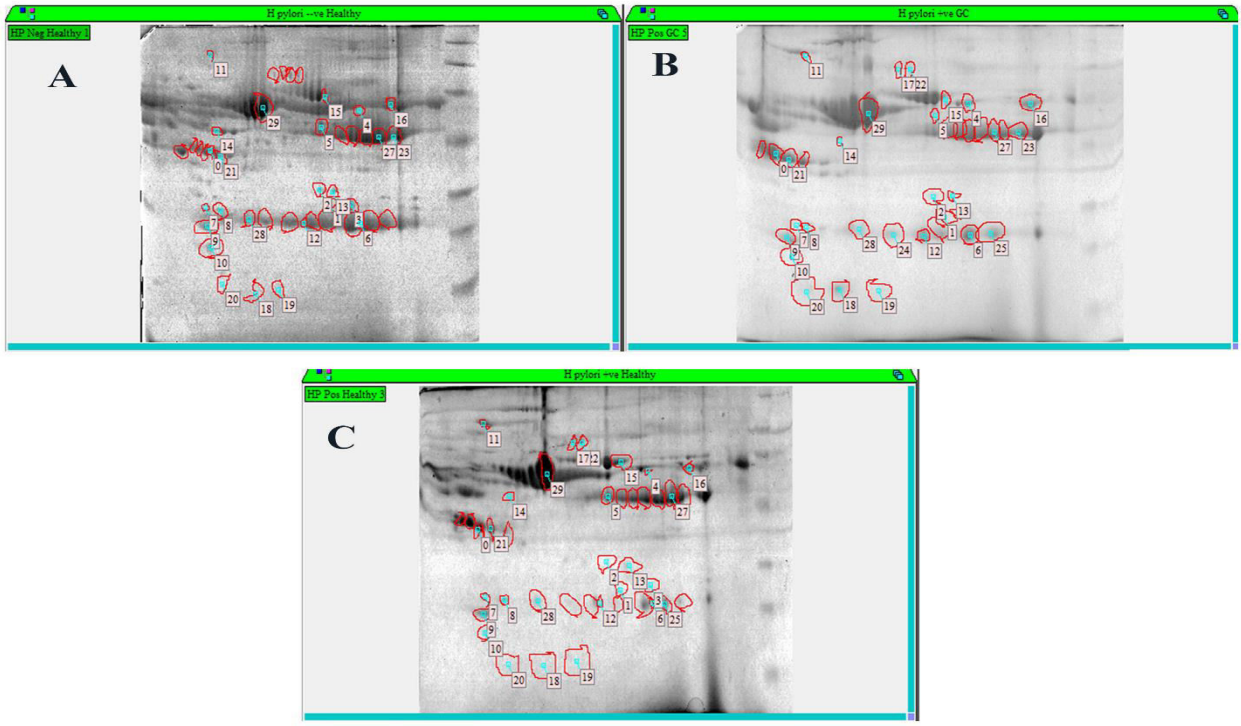

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Figure 2. The percentage of relative intensity of all the thirty protein spots were depicted in comparison with the serum samples (HP negative healthy, HP positive healthy, HP positive gastric cancer).

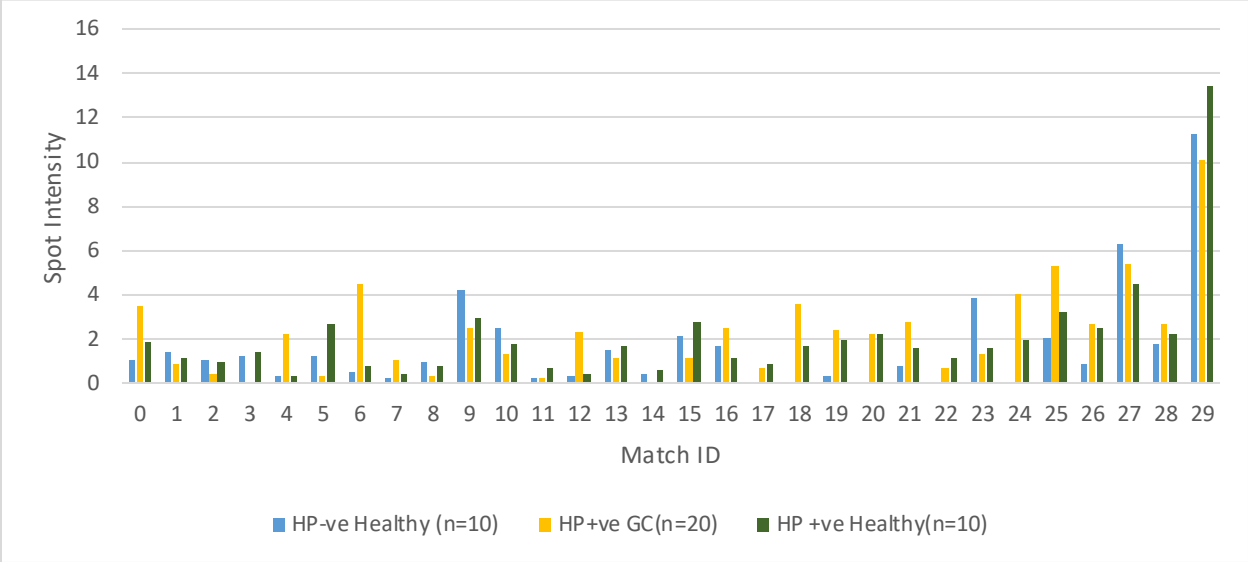

Figure 3(A-C).(A)Molecular Function, (B) Biological Process and (C) Cellular Component for the differentailly expressed proteins using PANTHER analysis

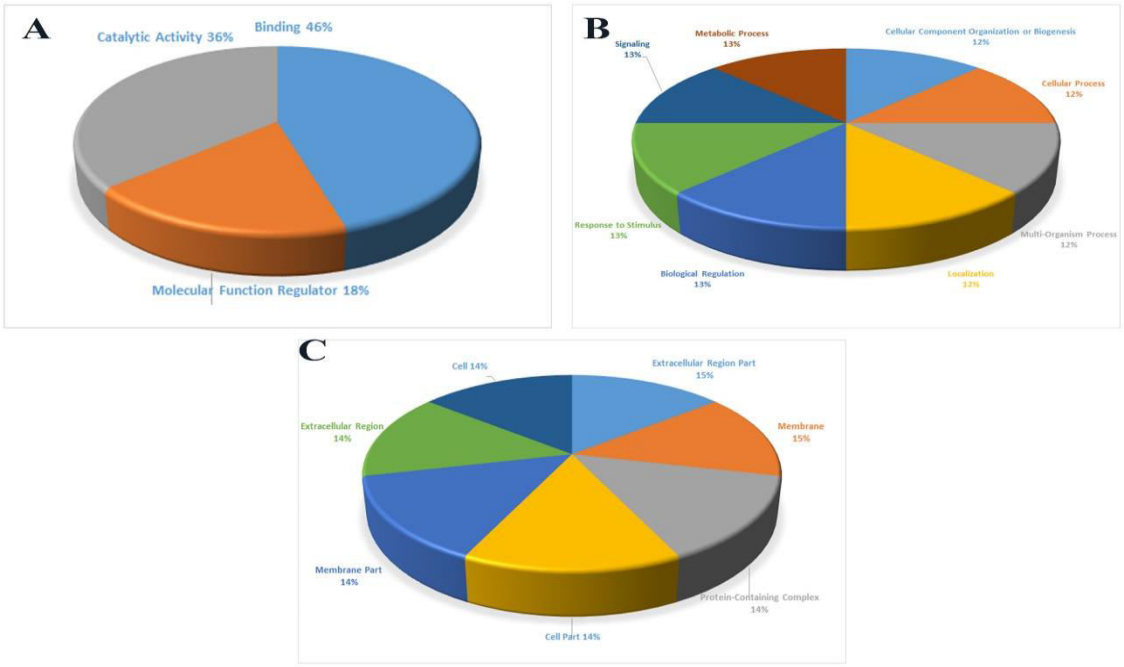

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Figure 4. Protein protein interaction analysis for the differentially expressed proteins using STRING analysis.

(Legend) (4A) Describes the pattern of interaction within the differentailly expressed protein – Complement factor and Immunoglobulin; (4B) Describes the pattern of interaction with Cluster analysis