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Comparative proteomic profiling of *H. pylori* induced Gastric Cancer Serum with healthy serum samples

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ABSTRACT:

Gastric Cancer (GC) is the third most common cause of death from cancer, accounting for about 7,83,000 deaths in males and females throughout the world. Therewere very few studies has been made on population specific biomarker studies withrespect to *Helicobacter pylori* (*H. pylori)* induced gastric cancer in India. Hence, the present study aimed toexecute this biomarker study involving positive and negative healthy serumsamples of *H. pylori* in comparison with *H. pylori* positive gastric cancer samples.

KEYWORDS:

Proteomics, Gastric Cancer.Serum, Protein Estimation, SDS PAGE,

INTRODUCTION:

In the Global scenario of Gastric Cancer, India falls under the low incidence category. In India, it is the fifth most common cancer amongst men and sixth most common in women [1]. It is the second most common cause of cancer related deaths among Indian men and women in the age between 15 and 44; it ranks among the top five most common cancers [2, 3,4]. Majority of the patients, present at an advanced stage at the time of first presentation itself. This directly translates into a decrease in 5-year survival rate compared to countries where early diagnosis is made, especially in countries where screening facilities are available.

Gastric adenomas, atrophic gastritis, *H. pylori* infection [5] and previous gastric surgeries are considered to be common risk factors across the world. *H. pylori* infection is considered to be one of the major risk factors for gastric cancer. In India, the general prevalence of *H. pylori* positivity is between 80 and 90% and gastric cancer is reported to develop in 0.1–3% of patients [6]. The association of *H. pylori* to gastric carcinogenesis is influenced by multiple factors which include the geographic location, dietary practices and other potential risk factor exposure. More so the dietary habits of the people keep dynamically changing based on their urbanization, social empowerment and improving economic status. The type of method used for *H. pylori* estimation and quantification variation could be a potential reason which one should exercise caution when interpreting these data.

The demanding priority at the moment is improvement in basic, clinical and translational research and skill transfer to set the optimum outcome in management of gastric cancer. Diagnosis of early gastric cancer in India continues to be a problem and routine screening is neither feasible nor cost effective considering the population and over all incidences. Biomarker discovery and the development of clinical diagnostic tests can improve the performances in early detection, clinical decision-making and clinical outcomes. The technological improvements in the field of proteomics opened new horizons for the discovery of novel biomarkers for many existing pathologies. The first important distinction that has to be taken into account is about biomarker discovery and validation. The biomarker discovery procedure is very demanding and requires, from the proteomics point of view, expensive equipment, very well-trained personnel, and precious specimens. Two-dimensional electrophoresis (2DE) and later 2D–DIGE play a key role in the bio- marker discovery field mostly because they probably still represent the best technique for the visualization of protein isoforms or proteoforms of abundant proteins that are valuable as biomarkers.

Proteomics aims to retrieve all the information present at the protein level in a given biological system. It requires the ability to separate and resolve all proteins in the system, track changes in protein expression and sub cellular location, identify and classify proteins into functional

groups. At present, two dimensional electrophoresis (2DE) gel based proteomics remains an important tool for achieving this goal. It is able to qualitatively and quantitatively compare protein profiles under different conditions to further explain biological systems and identify the disease markers that promise to become the diagnostic and pharmaceutical targets of the future [7]. Two-dimensional (2D) sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) was first described in 1975 and combined with mass spectrometry (MS) maintains its place as one of the most effective technologies for proteomic analysis [8, 9].

Appropriate sample preparation method optimized for a specific tissue is essential for protein separation in 2DE [10, 11, 12, 13]. Samples should have a high protein concentration and should be free of salt and other compounds, such as ionic detergents, nucleic acids, lipids, etc., since these could interfere in isoelectric focusing (IEF) and second dimension separation. An ideal protein extraction procedure should reproducibly recover the entire complement of proteins in solution with very minimal degradation and in parallel it should exclude contaminating non proteinaceous compounds [14]. Sample preparation methods however, often lead to protein modifications, which can affect the results of the proteomic study and in other cases might lead to protein loss. Several methods are reported for concentrating the proteins [15, 16, 17]. Efficiency of these methods differs in protein recovery and efficiency of salt removal.

For 2D analysis, rehydration & sample application method, pH range of IPG strips and separation distance are the essential factors to be optimized. The dry IPG strips have to be rehydrated to their original thickness of 0.5mm with rehydration buffer containing appropriate denaturants, reductants, detergents and carrier ampholytes [18]. Rehydrating dry strips with sample solubilised in rehydration buffer is termed as sample in-gel rehydration, which is "passive" if no voltage is applied and "active" if low voltage (30-50V) is applied for sample uptake. Active rehydration helps in efficient uptake of very high mol. wt., highly alkaline and hydrophobic proteins. However, "Cup loading" is the most reliable method for sample application after rehydration of the strips with sample free rehydration buffer first. Sample application could be at

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either anodic or cathodic end by cup loading [19]. This method is preferred for preparative gels in particular when high protein amounts have to be loaded in small volume.

The objectives focused in these sections were

a) Optimization of high-resolution two-dimensional electrophoresis for serum proteome analysis

b) Examination of the proteome profile of *H.pylori* induced gastric cancer serum and healthy serum samples.

RESULTS& DISCUSSION

Serum samples were optimized for two-dimensional gel analyses. Figure 1represents the optimized 2D gel using serum samples obtained from gastric cancer patient and healthy subjects. The differentially expressed protein spots were marked for better understanding and gel analysis. Healthy subjects were categorized into two based on their *H.pylori* status, three were showing positivity for *H.pylori* infection and three were showing negativity for *H.pylori* infection. Hence the comparison was made in both the condition. Among the serum sample obtained from gastric cancer patients, five showed positivity for *H.pylori* infection and one sample showed negativity for *H.pylori* infection. Only the samples which showed positivity for *H. pylori* infection were taken for two-dimensional gel electrophoresis and mass spectrometry analysis.

Figure 1: 2D gel electrophoresis of HP negative healthy serum samples separated with pI range of 3-10 by adapting active rehydration method. The 2D gels were stained with CBB

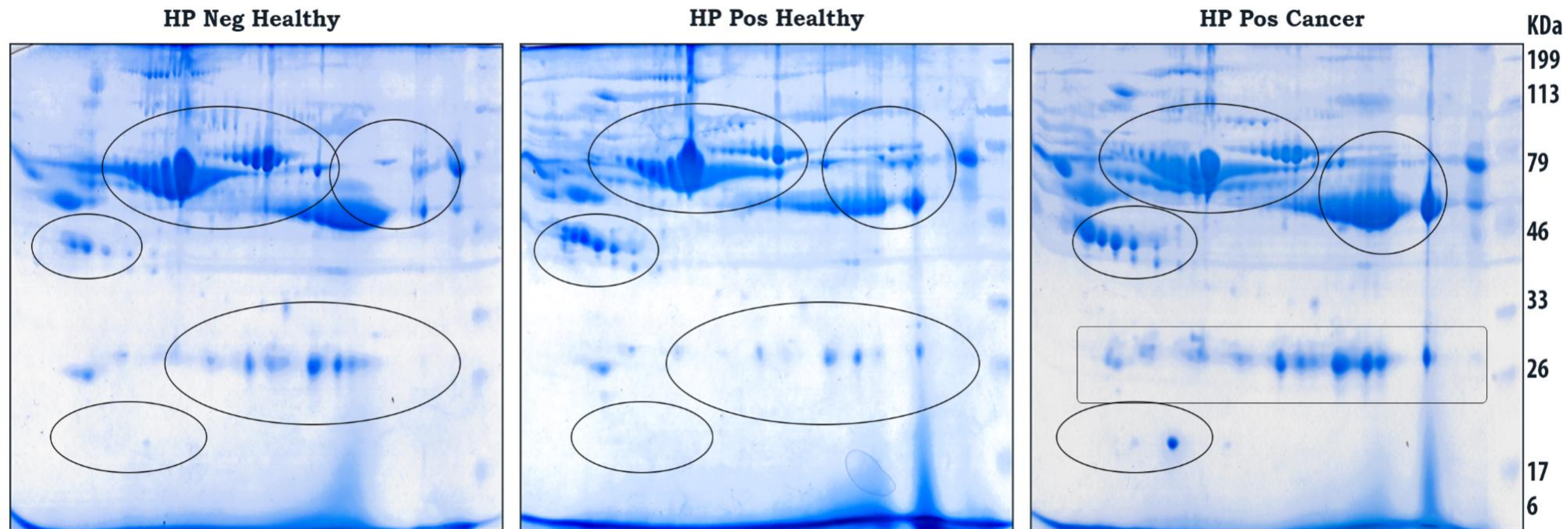

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Figure 2: 2D gel electrophoresis of HP positive healthy serum samples separated with pI range of 3-10 by adapting active rehydration method. The 2D gels were stained with CBB 250 overnight and destained with milli Q water.

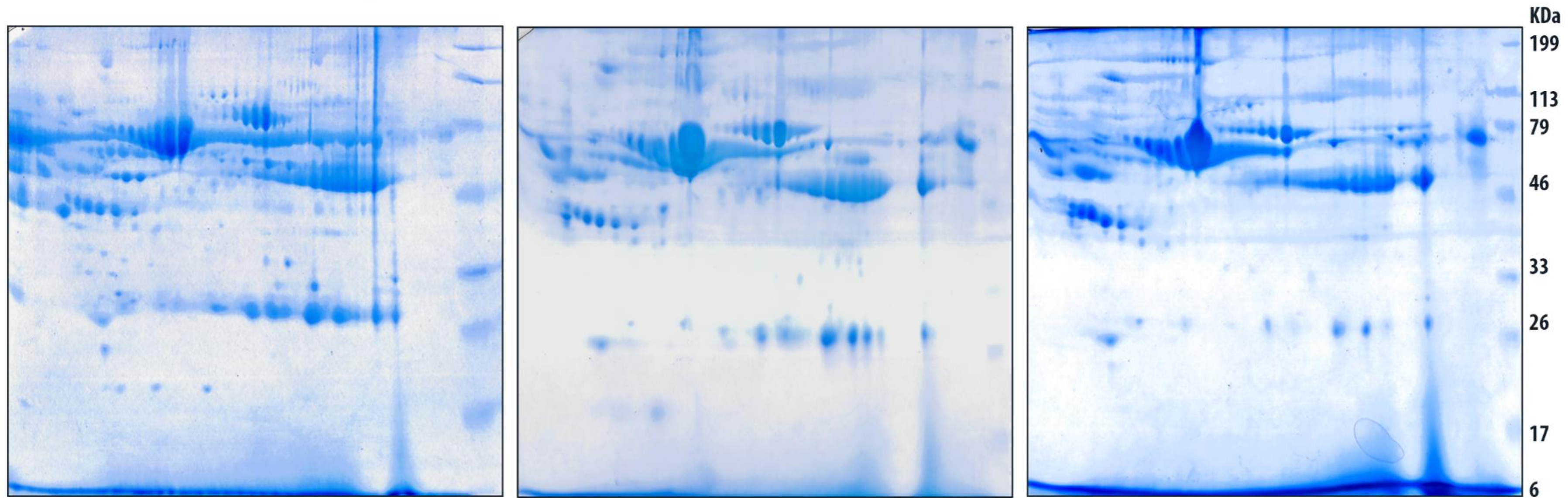

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Figure 3: 2D gel electrophoresis of HP positive gastric cancer serum samples separated with pI range of 3-10 by adapting active rehydration method. The 2D gels were stained with CBB 250 overnight and destained with milli Q water.

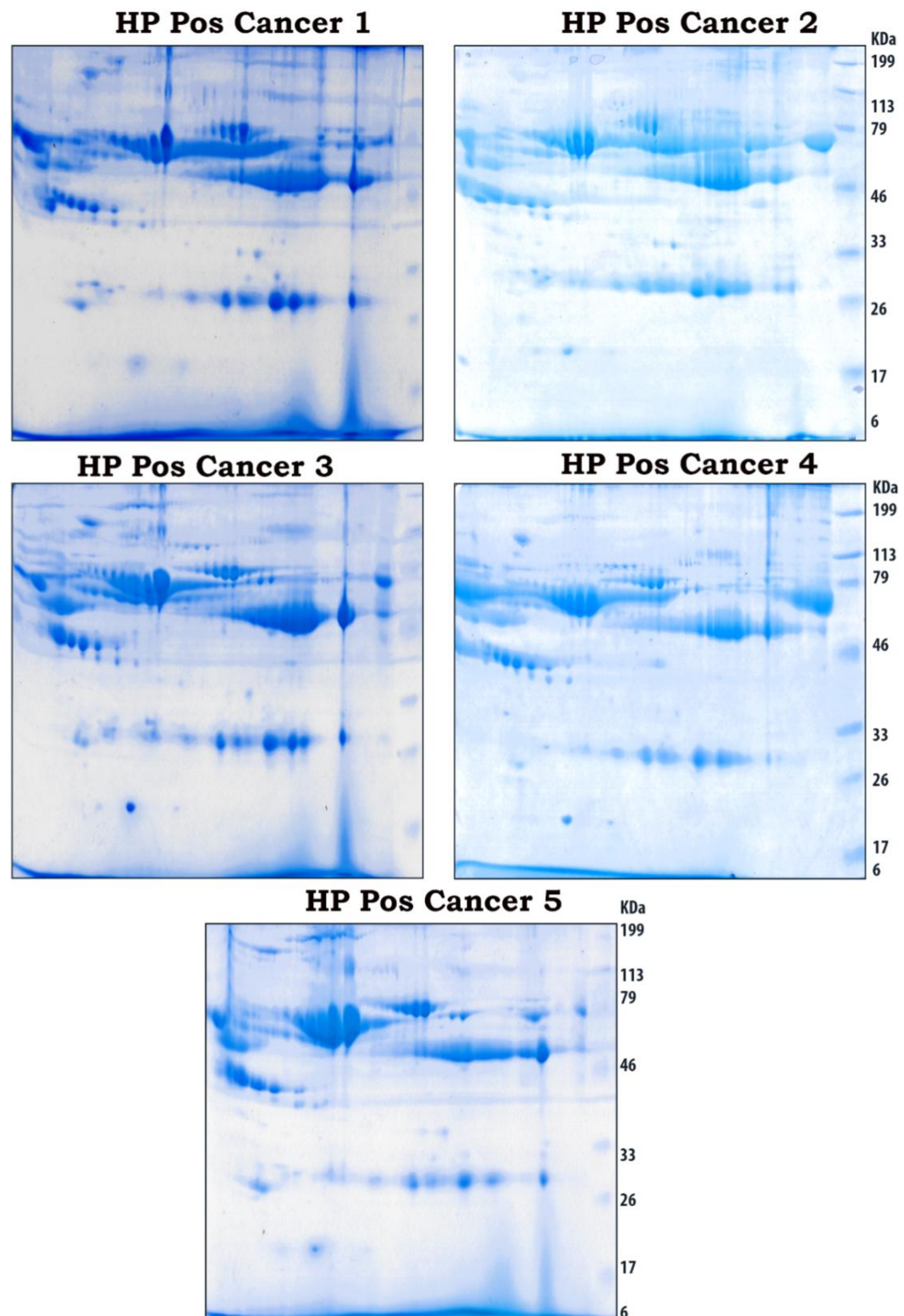

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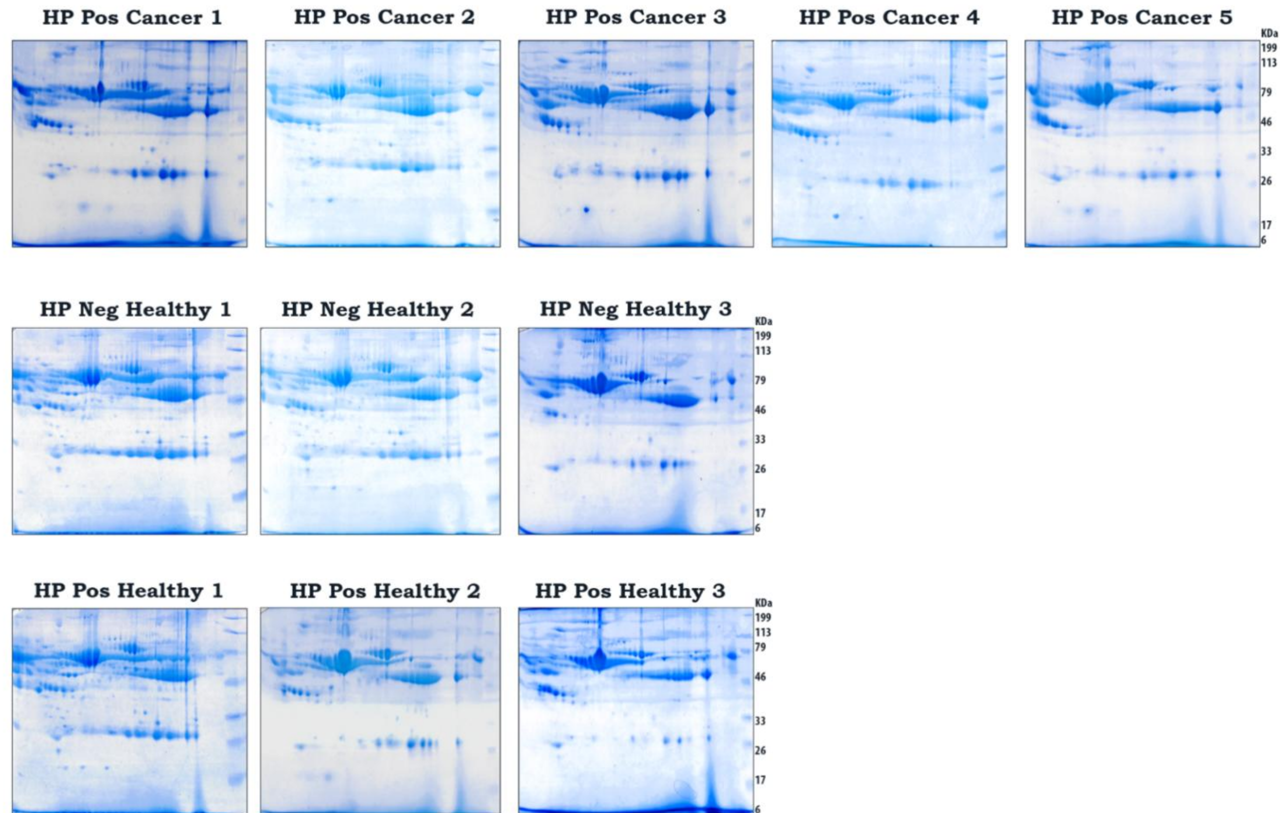
Figure 4: 2D gel electrophoresis of all the serum samples (HP negative healthy, HP positive healthy, HP positive gastric cancer) separated with pI range of 3-10 by adapting active rehydration method. The 2D gels were stained with CBB 250 overnight and destained with milli Q water.



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CONCLUSION

In our study, we found that the figure 1shows all the healthy serum samples which were negative for *H.pylori* infection and figure 2 represents all the healthy serum samples which were positive for *H.pylori* infection. With respect to serum samples from gastric cancer patients, all the five samples which were positive for *H.pylori* infection were analysed by 2D gel electrophoresis and represented in figure 3. Figure 4 illustrate the complete serum sample set including HP negative healthy, HP positive healthy, HP positive gastric cancer which were adapted and analysed using 2D electrophoresis for this study.

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