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Qualitative Analysis of Serum Proteins in Oral Cancer Separated by SDS-PAGE

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ABSTRACT

This study was conducted for qualitative estimation of serum proteins separated by SDS-PAGE in order to describe the definition of potential serum proteins that may act as diagnostic marker in oral cancer. Serum samples of 25 biopsy confirmed cases of oral cancer and normal controls of similar age group were subjected to SDS-PAGE on a 12% resolving gel, followed by staining with Coomassie Brilliant Blue R-250. Protein fractions were analyzed using computer software program "Gene Genius Gel Documentation and Analysis System". Major protein fractions ranging in molecular weights from 1.46-159 kDa were observed. Raw volumes of most of the protein fractions seem to be increased in majority of oral cancer cases as compared to normal control. Protein fractions 56-58 kDa were undetected in normal controls under 80 years of age but appeared in 54% of Oral cancer patient. Further advancement of this work could yield better resolution of protein fraction 56-58 kDa that could serve as marker for oral cancer.

Keywords: oral cancer, serum protein, analysis, SDS-PAGE.

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INTRODUCTION

Oral cancer is a heterogeneous group of cancers arising from different parts of the oral cavity, with different predisposing factors, prevalence, and treatment outcomes. It is reported that frequency of this cancer is 6th in men and 14th in women.¹ Tobacco smoking and alcohol drinking are recognized as major causative agent of cancer of the oral cavity.^{2,3}

It has been observed that different age group also affect the oral cancer. some study show that more than 90 percent of oral cancer cases are reported in person above of 45 years.60years is the average age. Four decades before the ratio of oral cancer in male to female was 5:1 but now it is 2:1. the increased rate of smoking in women is responsible for this drastic change.⁴ During this cancer these parts of oral cavity may affected such as lip, tongue, gingiva, oral mucosa, oropharynx, and hypopharynx.⁵ The exact prediction of oral cancer may be achieve by preparation of specific biomarkers.⁶

Proteins play a key role in cell functions and pathways, identifying differentially expressed proteins between normal and pathological state, leading to a better perceptive of the cellular mechanisms involved in disease. Some proteins are down-regulated and others are up-regulated with the onset of disease, depending on a protein's specific function, undergoing disease-specific posttranslational modifications.⁷⁻⁹ This identification of changes in protein profile that occur during pathological state could lead to the discovery of protein biomarkers and novel strategies for the enhancement of detection and diagnosis of oral cancer without any unfairness. This will also minimize unnecessary biopsies.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a adaptable and powerful technique widely used for protein separation based on their molecular weights.¹⁰ In this study protein fractions were studied by SDS-PAGE due to its importance in the declaration of serum proteins. In this study the aim of performing SDS-PAGE was to make a comparison between quality of serum found in healthy subjects and OC cases and to identify some novel proteins that are raised or lowered in concentrations in diseased subjects.

MATERIALS AND METHODS

Sample source and Patients

This study was conducted at CHRI a regional cancer centre for research and treatment Gwalior M.P, India and College of life sciences, Jiwaji University. Gwalior M.P, India. Patients who had undergone any surgical intervention of OC were excluded. Biopsy confirmed OC cancer patients were also excluded from study.

Blood samples of 25 biopsy confirmed OC patients of different ages were collected from pathology units of Regional Cancer center Gwalior. Normal healthy males matched by age were also included from general community as control group. Similarities of their basic confounding factors were ensured. The samples were distributed into four groups according to age. (Table.1) Serum were separated and stored at -20°C till analysis.

Table.1: Samples distribution

S.No	Group	Age Range	Sample Classification
1	I	50-59	T ₁ -T ₆ (Six OC case) +N ₁
2	II	60-69	T ₇ -T ₁₂ (Six OC case) +N ₂
3	III	70-79	T ₁₃ -T ₁₇ (Five OC case) +N ₃
4	IV	>80	T ₁₈ -T ₂₃ (Six OC case) +N ₄

T- Test number, N –Normal control

Sample preparation and SDS-PAGE separation

Sample was prepared in the ratio 1:1 with sample buffer. Proteins were resolved by 12% SDS-PAGE using PROTEAN one-dimensional electrophoresis (Bio-Rad, Hercules, CA, USA) .Since equal quantities of protein from each sample under comparison must be loaded on to gels for electrophoretic analysis, so the total protein content of each serum sample (cases and controls) was estimated by Lowry Method (1951) assay ¹¹.

In each group serum samples of control and OC cases were separated by loading in their respective wells. The gel was then electrophoresed at constant supply of 12mA and voltage of 150V in a minicold lab maintained at 4°C . The gel was stained using Coomassie Brilliant Blue R-250 and then destained. Protein fractions appeared as dark bands on a light background. Gels were photographed using “GeneSnap” and their images were formatted and analyzed by using “GeneTools”, which were part of the computer software program “GeneGenius Gel Documentation and Analysis System”. The molecular weight of each protein fraction was determined by using molecular weight markers as standard. The raw volume was calculated by the software using band height and intensity, which was a measure of relative quantity of protein in each sample.

RESULTS AND DISCUSSIONS

Protein biomarkers are very useful for diagnosis and prediction of various forms of different cancers and other diseases. Certain proteins can be up or down regulated during disease development. Detection of these variations in protein expression levels, as a function of disease development is important for prediction. To distinguish between normal and pathological state of OC, a qualitative analysis of Coomassie Brilliant Blue-stained proteins separated by SDS-PAGE

was used in this study. This procedure yielded 14 major protein fractions ranging in molecular weight from 1.46-158 kDa, their appearance and raw volume in serum sample of each case was, studied (Figures, 1 to 4) .

In group I (**Figure-1**) (50-59 years) protein fractions ranging in molecular weight from 1.46-158 kDa were observed when samples were subjected to SDS-PAGE. Raw volume of most of the protein fractions in this group were increased in majority of OC cases, while some proteins were decreased as compared to normal control (N_1). In one sample (T_3), it was observed that most of the major protein fractions (158, 140, 132, 122, 114, 100 and 76) were undetectable and remaining was decreased in raw volume when compared to other cases and normal control in same group. Furthermore raw volumes of all protein fractions were also down regulated in sample (T_2). Protein fractions 56-58 kDa molecular weights were also found absent in the control but present in two of OC cases (T_1 , T_5).

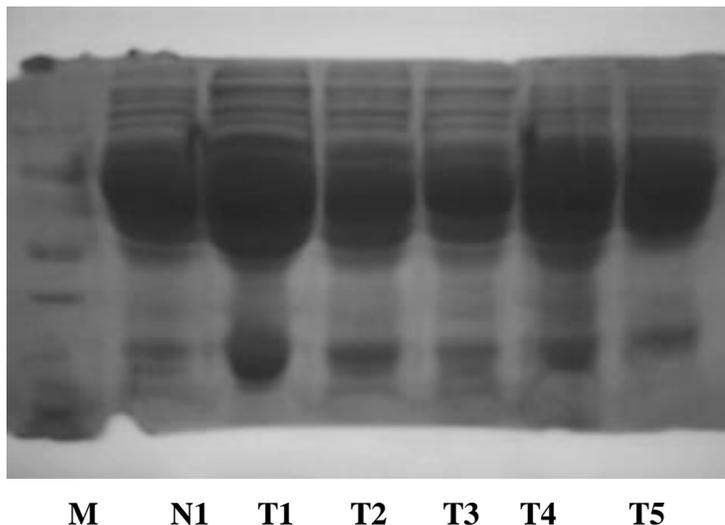
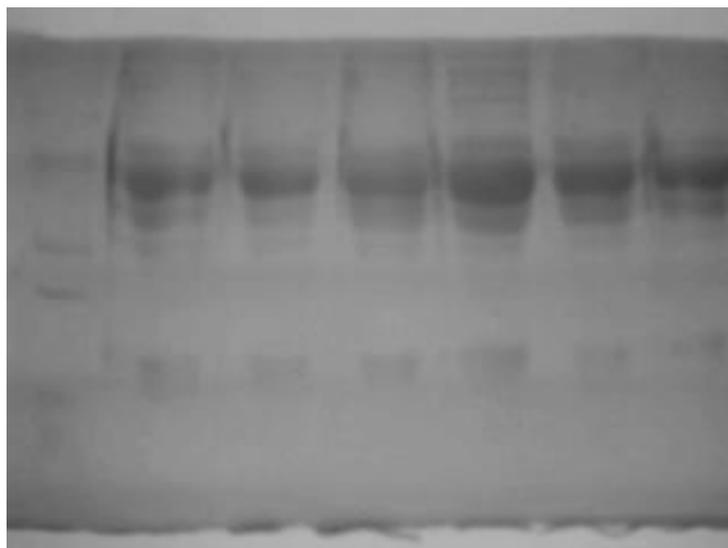


Figure-1 Serum protein profile of control (N_1) and cases (T_1 - T_5) in group I (50-59 yr.), resolved on 12% resolving Gel by SDS-PAGE.

M: Protein size markers (from top to bottom): 67, 45, 24, 18, 13 and 1.46 kDa.

In group II (**Figure-2**) (60-69 years) when samples of OC cases and normal control were subjected to SDS-PAGE, it showed same patterns as in group I. Raw volume of protein fractions were up regulated. Exceptional behavior was observed in two samples (T_9 and T_{11}), in which protein fractions of molecular weight 132, 122, 114 and 100 kDa, appearing in all the other samples were absent and remaining fractions were decreased in raw volumes. Like group I, protein fraction of 56-58 kDa was also found absent in control (N_2), but it was seen in OC cases i.e. T_7 , T_8 and T_{10} of group II.



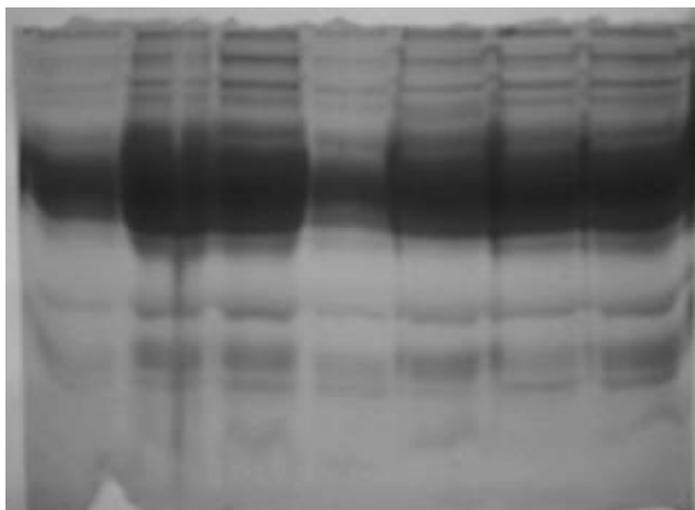
M N2 T7 T8 T9 T10 T11

Figure-2. Serum protein profile of control (N₂) and cases (T₇-T₁₁) in group II (60-69 yr.), resolved on 12% resolving gel by SDS-PAGE.

M: Protein size markers (from top to bottom): 67, 45, 24, 18, 13 and 1.45 kDa.

Unlike group I and II, group III (**Figure-3**) (70-79 years) showed an overall increase in raw volume of protein fractions in all OC cases (T₁₃-T₁₇) as compared to normal control (N₃).

Whereas similar to normal controls in group I and II protein fractions 56-58 kDa was absent in the control (N₃) but appeared in T₁₅ and T₁₆ OC cases in this group.



M N3 T13 T14 T15 T16 T17

Figure-3. Serum protein profile of control (N₃) and cases (T₁₃-T₁₇) in group I (70-79 yr.), resolved on 12% resolving gel by SDS-PAGE.

M: Protein size markers (from top to bottom): 67, 45, 24, 18, 13 and 1.46 kDa.

In group IV (**Figure-4**) (>80 years), the raw volume of most of the protein fractions was found to be increased in OC cases relative to the control (N₄). Anomalous behavior in this Group was displayed by one sample (T₂₁), in which four protein fractions of molecular weights 132, 122, 114 and 100 kDa were not seen. The 114 kDa fraction was also found absent in two samples (T₂₀ and T₂₂). Protein fractions 52-57 kDa was seen in control (N₄) of this group which was found absent in normal controls of all other groups. These protein fractions were also seen in all OC case (T₁₈-T₂₂) of this group.

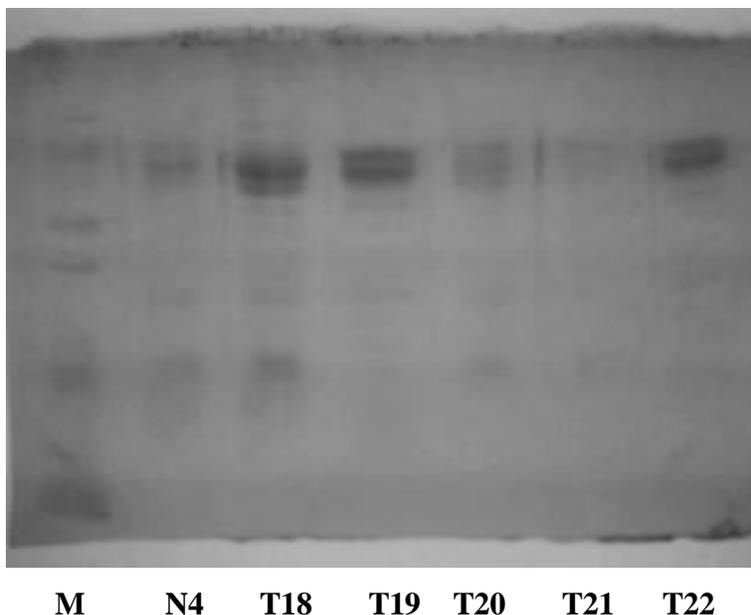


Figure-4. Serum protein profile of control (N₄) and cases (T₁₈-T₂₂) in group I (> 80 yr.), resolved on 12% resolving gel by SDS-PAGE.

M: Protein size markers (from top to bottom): 67, 45, 24, 18, 13 and 1.46 kDa.

On the whole, it was observed that raw volume of most protein fractions was increased in majority of cases as compared to controls. In the present study protein fractions 56-58 kDa appeared in 54% of OC cases (T₁, T₅, T₇, T₈, T₁₀, T₁₅, T₁₆ and T₁₈-T₂₂) and was unnoticeable in normal controls under the age of 80 years. But above 80 years was observed protein fractions 56-58 kDa. Similar findings was observed in sub cellular concentration of proteins in normal and OC cancer cases. They found significantly higher concentrations of non- Histone proteins of 43 KD, 55KD and 192 KD in OC cases than in the normal controls and their results also showed that contents of other major protein species were similar in specimen of normal and OC cases.¹² the possibility of rising oral cancer increase with age but about 1 out of every 3 people diagnosed are younger than 55.⁴

Serum samples were used in present study to compare the protein profiles among study groups. This study also revealed that there is 29% increased of protein fraction 57 kDa in of OC than normal controls.

Previous studies have shown that the concentrations of most of the proteins are too little to be detected by Coomassie Blue staining. However, these proteins could not consider “unique” to their respective samples, because amount of proteins that did not exceed the minimum sensitivity of the dye may have been present in other samples.¹³ In the present study, SDS-PAGE instead of 2D-PAGE and Coomassie Brilliant Blue R-250 in place of silver staining was used; therefore, amount of protein fractions seen was much lesser. As quoted above, the protein fractions of 56-58 kDa, which were unnoticeable in the controls N₁, N₂ and N₃ and was present in their corresponding OC cases, cannot be considered totally absent in these controls as well as other OC cases, because amounts of protein that did not exceed the minimum sensitivity of the dye could be present.

This study also informed regarding peculiar behavior of few samples of different age group like T₂, T₃, T₉, T₁₁ and T₂₁. The major protein fractions were either unnoticeable or down regulated in these samples. Protein fractions of with molecular weights 132, 122, 114 and 100 kDa were absent in T₃, T₉, T₁₁. In addition to this, protein fraction 114kDa was also absent in sample T₂₀ and T₂₂. These results strength be evident of their malignant transformation although further study of this work is necessary by other sophisticated method.

CONCLUSIONS

Further examination of these samples could yield more information; silver staining, which is more sensitive than Coomassie Blue. Two dimensional gel electrophoresis would separate the proteins according to ionic potentials as well as molecular weight and thus achieve better resolution. Comparison of serum protein profiles in oral cancer with normal controls can give an insight for the improvement in detection and diagnosis of OC without any discrimination and unnecessary biopsies. This conclusion plays a significant role in demonstrate that serum enriched specific biomarker which may be reliable source for testing.

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