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STUDIES ON SERUM PROTEIN PROFILES AS BIOMARKER OF CANCER

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ABSTRACT
Serum being a good source of variety of proteins secreted by different cells has been taken for protein profile demonstration from different cancer patients and examined by SDS-PAGE analysis. The band patterns of serum samples, after comparison with that of normal healthy people as control, revealed that serum sample from cancer patients have extra protein bands which were absent in control. Molecular weight of extra protein bands in test serum sample has been calculated with the help of protein molecular weight markers and discussed.

INTRODUCTION

Alteration in gene(s) of a normal cell i.e. mutation is responsible for initiation of cancer. Cancer is genetic in origin and starts from somatic mutation (Sinha *et al.,* 1998)

Cancer is an autonomous and abnormal proliferation of cells either invade the adjacent tissue (invasion) or migrate to distant sites (metastasis). Cancer cells are altered self cells that have escaped normal growth regulating mechanisms by a series of acquired or inherited mutations damaging genetic information. Cancer, the biggest killer next to heart disease has multifactorial aetiology viz. direct genotoxic effect on DNA,hormone induced cell proliferation,induced oxidative damage of DNA (Kasai *et al.,* 2009), ROS induced DNA damage (Ferk *et al.,* 2009). The development of cancer appears to induce the activation or the altered expression of oncogenes (Aaronson, 1991), the loss or inactivation of tumor suppressor genes or both.

Due to severity of the disease attempts have been made to dectect and cure cancer from different angles. One of the many ways being tried for diagnosing cancer is use of biomarker.

An ideal biomarker should be both specific for a given type of cancer and sensitive enough to detect small tumors for early diagnosis during screening. Unfortunately most tumor markers known today are neither specific nor sufficiently sensitive for such purposes.

A large number of the proteins of our body are present in serum whose expression is regulated by proto-oncogenes (e.g. signal transduction molecules, growth factors, growth factor receptors etc.) and their expression is abnormally high during oncogenesis. Many of these proteins are unexplored till now. Advances in genomics, proteomics and molecular pathology have generated many candidate biomarkers with potential clinical valu (Ludwig and Weinstein, 2005). Alexandrov *et al.*, (2009) through serum protein profiles as biomarker, using discrete wavelet transformation, provided a high recognition rate (97.3%), sensitivity (98.4%) and specificity (95.8%) of cancer.Systematic researches for plasma proteins that are biological indicators, or biomarkers, for cancer are underway(Hanash *et al.,* 2008). In recent years use of proteomic technologies for identifying the set of proteins that are related to the disease are being tried (Montazery *et al.,* 2008). The present communication deals with examination of various protein typesfrom serum found in different carcinogenesis as biomarker.

MATERIALS AND METHODS

Collection of blood samples: The blood samples were collected from patients suffering from different types of cancer and named according to the type of cancer like G.B. = Gall bladder cancer samples; B.C. = Breast cancer samples; Cc. = Cervix cancer samples; Lx. = Larynx cancer

samples; B. M. = Buccal mucosa Cancer samples; O. V. = Ovary cancer samples; L. U. N. = Lung cancer samples; B. O. T. = Base of Tongue cancer samples and petientnumber was given.

Preparation of serum from collected blood: 5 mL. of collected blood sample was incubated for one hour at room temperature, and the unclotted portion of the blood was centrifuged at 2000 rpm for 10 minutes. The supernatant was collected into an eppendroff or microfuge tube and stored at - 20^{0}C. This supernatant (serum) was used for experiments. Control serum was prepared from the blood of normal healthy person.

SDS-PAGE of the prepared serum samples: SDS-PAGE of different test serum samples was performed (Laemmli, 1970). Concentration of the protein sample before PAGE was calculated spectrophotometrically by using standard protocol (Campbell, *et al*., 1984).

Determination of molecular weight of protein: In order to know the molecular weight of extra protein band (s), medium range protein molecular weight marker (Bangalore Genei- PMWM) was used in which PMWM have seven proteins of known molecular weight (Table 1). Rf value of these known molecular weight proteins were calculated.

Regression curve of Rf value (X-axis) against $\log_{10}$ molecular weight (Y-axis), was plotted to get a standard curve for determination of molecular weight of unknown proteins (Fig. 1)From trend line the equation was derived as Y = - 1.447 X + 2.027.

RESULTS AND DISCUSSION

Cancer is an abnormal proliferation of cells of particular origin (localized tumors) or cells from different origin (generalized tumor) due to activation of some proto - oncogenes leading to the production of some abnormal proteins which are generally absent in normal healthy persons. The abnormal proteins may be a growth factor, growth factor receptor, intra-cellular signaling molecule

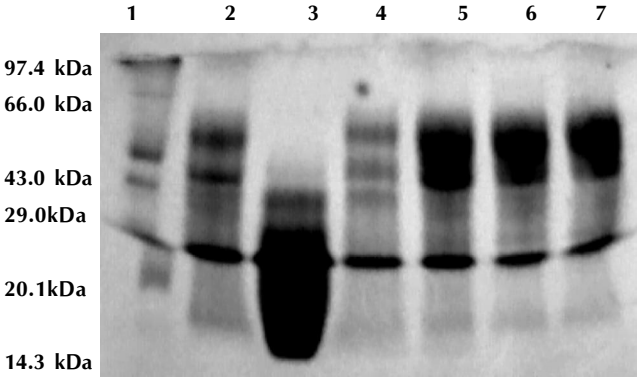

Figure 1: SDS-page protein profile in different types of carcinoma. 1= Marker; 2 = GB99; 3 = BC60; 4 = Cc56; 5 = LX95; 6 = BM29; 7 = CONTROL

Table 1: Different marker proteins, their molecular weight and Rf value

Marker proteins	KDa	Log_{10} (M. W.)	Rf value
Phosphorylase b	97.4	1.9886	0.040
Bovine serum albumin	66.0	1.8195	0.100
Ovalbumin	43.0	1.6335	0.320
Carbonic anhydrase	29.0	1.4624	0.400
Soyabeantrypsin inhibitors	20.1	13010	0.500
Lysozyme	14.3	1.1523	0.580

or enzyme of different signaling pathways and can be taken as marker for diagnosis of tumor in early stages, for proper treatment and cure.

SDS PAGES run with different combinations of test serum samples. In all cases controls were taken from serum of same normal healthy person.

The test serum samples showed marked variation in comparison to the control. In some samples extra protein bands were observed which were absent in control samples, while in others some protein bands were absent which were normally present in control serum sample.

An extra band was found between 29 kDa and 20.1 kDa bands in test serum samples of breast and cervix cancer but absent in other test serum samples (G.B., Lx. and BM)

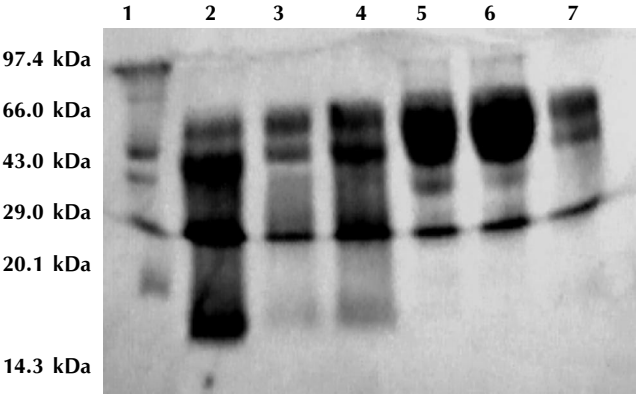

Figure 2: SDS-page protein profile in different types of carcinoma. 1= Marker; 2 = GB106; 3 = LX 90; 4 = OV100; 5 = LUN20; 6 = BOT16; 7 = CONTROL

and control. The molecular weight of this band protein has been estimated to be approximately 27 kDa (Fig. 1).

The test serum samples of LUN and BOT also showed an extra band between 29 kDa and 20.1 kDa bands was absent in other test serum samples (G.B., Cc and OV). Molecular weight of this protein band which has been estimated to be also approximately 27 kDa (Fig. 2). Test serum samples of G.B. Cc (very low) and OV showed an extra band after14.3 kDa band. The molecular weight of this band protein was approximately 14 kDa (Fig. 2). The test serum samples of breast cancer also revealed an extra 14 kDa band (Fig. 3)

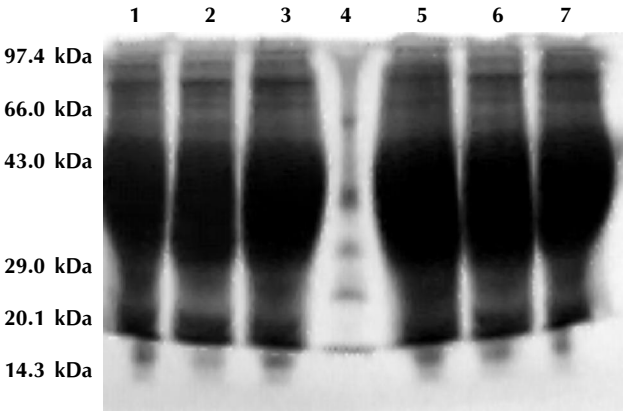

Figure 3: SDS-page protein profile in breast carcinoma. 1 = BC2; 3 = BC12; 4 = Marker; 5 = BC60; 6 = BC63; 7 = Control

The 27 kDa proteins (expressed in G.B., Lx, Cc and OV) and 14 kDa protein (expressed in G.B., Cc, OV and B.C.) might be carcinogenic which is not expressed in control. The generalization and further confirmation of these bands may lead to use it as potential tumor marker in future for early detection of different cancers.

In earlier studies a mysterious heterogeneity of results based on proteomics for any individual patient of cancer apparently equivalent type, stage and grade (Ludwig and Weinstein, 2005) has been reported. Those differences in outcome may relate, in part, to stochastic events, such as the time at which a single cancer cell happens to undergo all of the steps necessary for successful metastasis, or they may relate to factors that can be reasonably well understood at a deterministic level. Even if molecular markers connot eliminate the stochastic uncertainties and enable us to predict outcome definitively, they will almost certainly increase our accuracy at sub-classifying patients and their cancers. Alexandrov *et al.,* (2009) while working on biomarker discovery in MALDI-TOF serum protein profiles using discrete wavelet transformation reported that the

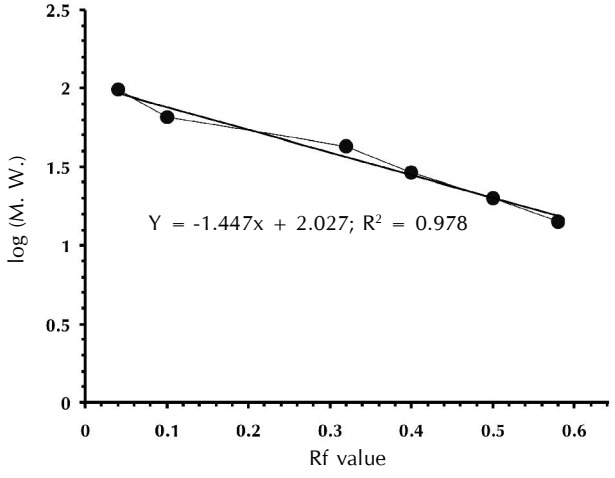

Figure 4: Standard curve for molecular weight determination by SDS - page

extracted biomarker patterns mostly represent the peaks expressing mean differences between the cancer and control spectra. However, we showed that the discriminative power of a peak is not simply expressed by its mean height and con not be derived by comparison of the mean spectra. The obtained classifiers have high generalization power as measured by the number of support vectors. This prevents overfitting and contributes to the reproducibility of the results, which is required to find biomarkers differentiating cancer patients from healthy individuals. The difficulties caused by the complexity of biological-fluid proteomes (which contribute proteins to plasma) and by the extensive heterogeneity among diseases, subjects and levels of sample procurement are gradually being overcome (Hanash *et al.,* 2008) The variation in protein band may be a result of such heterogeneity in cancer stage, grade or individuality of the patient.

As the serum proteins are extra cellular in nature the presence of abnormal (extra) protein bands in the test serum sample may be due to some activity other than normal protein production which can be identified and used diagnosis.

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REFERENCES

Aaronson, S. A. 1991. Growth factors and cancer. *Science.* **254:** 1146-1153.

Alexandrov, T., Decker, J., Mertens, B., Deelder, M. A., Tollenaar, R. A. E. M., Maass, P. and Thiele, H. 2009. Biomarker discovery in MALDI – TOF serum protein profiles using discrete wavelet transformation. *Bioinformatics.* **25(5):** 643-649.

Campbell, I.D. and Dwek, R.A. 1984.Biological Spectoscopy. Benjamin Cumminga. Menlo Park.

Ferk, F., Chakraborty, A., Simic, T., Kundi, M. and Kansmuller, S. 2009. Antioxident and free radical scavenging activities of sumae (*Rhus coriaria* L.) and identification of galic acid and its active principle. Abstract in international symposium on Genomics and molecular basis of human disease (March 17 - 19, 2009) organized by Magadh Univ. Bodh Gaya. p. 16

Hanash, S. M., Pitteri, S. J. and Faca, V. M. 2008. Review article mining the plasma proteome for cancer biomarkers. *Nature.* **452:** 571-579.

Henderson, B. E., Ross, R. K., Pike, M. C. 1991. Toward the primary prevention of cancer. *Science.* **254**: 1131-1138.

Kasai, H., Kawai, K. and Li Yun – Shan. 2009. Analysis of 8 – hydroxydioxygluanosine as a marker of oxidative stress. Abstract in international symposium on Genomics and molecular basis of human disease (March 17 -19, 2009) organized by Magadh Univ. Bodh Gaya. p1.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriphage T4. *Nature*. **227:** 680-685.

Ludwig, J. A. and Weinstein, J. N. 2005. Biomarkers in cancer staging, prognosis and treatment selection. *Nat. Rev. Cancer*. ***5*(11):** 845-856.

Montazery-Kordy, H., Miran-Baygi, M. H. and Moradi, M. H. 2008. A data-mining approach to biomarker identification from protein profiles using discrete stationary wavelet transform. *J. Zhejiang Univ. Sci. B*. **9(11):** 863-870.

Sinha, M. P. 2009. Hepatocancinogenesis in wister albino rats through environmentally occurring carcinogen. Abstract in international symposium on Genomics and molecular basis of human disease (March 17 -19, 2009) organized by Magadh Univ. Bodh Gaya. p10.

Sinha, K. Singh, S. R., Gorai, A. C. and Sinha, M. P. 1998. Histological changes during induced hepatocarcinogenesis in wister albino rats through environmentally occurring carcinogen. In: Recent Advances in Ecobiological Research Vol. II. M. P. Sinha (Ed.) Asish Publishing Corporation. New Delhi. Pp. 437-456.