Original Article

SEPARATION OF CANCER PROTEINS USING POLYACRYLAMIDE GEL ELECTROPHORESIS

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ABSTRACT

Normally the cancer development occurs due to unregulated body metabolisms, Mutations, uncontrolled cell growth, some of the proteins also involved in development of cancer disease. The variation also occurs in the amount of albumins and globulins from a normal cell to cancerous cell. The present work is conducted to know the amounts of serum albumins and globulins in the cancer patients, for this, we selected positive cancer samples from patients admitted in Warangal district hospital and subjected to Organic solution precipitation and salt precipitation. Subsequently these precipitated protein samples were analyzed by SDS Polyacrylamide gel electrophoresis, and different bands of albumin and globulin seen with different concentrations in a densitometer.

Key words: *Plasma proteins, α β γ Globulins, Precipitation, Coomassie blue staining, SDS-page, Densitometer.*

INTRODUCTION

Cancer is largely mostly resulting from genetic mutations [1]. There have been numerous advances in understanding of the pathogenesis of cancer[2]. A normal cell turns into a cancer cell because of one or more mutations in its DNA, which can be inherited or acquired [3]. The development of cancer is a complex multistage process, involving not only genetic change, but also due to hormonal changes, co-carcinogen and tumor promoter effects such as activation of proto oncogenes to oncogenes and the inactivation of tumor suppressor genes[4]. These changes are result of point mutations, gene amplification or chromosomal translocation, often due to the action of certain viruses or chemical carcinogens[5]. Many components changes occur patients, importantly albumin and globulin level. The present study is designed to find out changes in protein level in cancer patients by using gel electrophoresis.

MATERIAL AND METHODS

Two positive blood samples of colorectal cancer and breast cancer have taken from cancer patients at Warangal district cancer hospital. The serum isolated from blood by the application of centrifuge, after the isolation the proteins present in serum has detected. The isolation of proteins involves the appropriate methods i.e. identification of proteins by electrophoresis following fractionation of human blood plasma. Normally plasma contains albumin, fibrinogen and a variety of globulins which can be separated and identified by electrophoresis & their normal conc. [Table-1]. The proteins can be fractioned by precipitation with salts or organic solvents and electrophoresis is used to scrutinize the separation[6]. Sodium sulphite is used as the salt as this is very effective at room temperature, this salt has advantage that it does not interfere with the assay of protein by the Biuret or Folin Lowry method unlike the more commonly used ammonium sulphate[7]. Methanol is a convenient organic solution as this precipitates the globulins with little or no deneturation[8]. For the fractionation of the proteins required following solutions such as sodium sulphite-12.6gm/100ml, sodium sulphite-15.8gm/100ml, sodium sulphite 21gm/100ml, human blood plasma, methanol: water (3:2), citrate buffer (0.1 mole/ lit, pH 6.8), sodium hydroxide-1mole/ lit, sodium chloride-0.9gm/100ml.

Method for salt precipitation[9]

0.5 ml of plasma added to 9.5 ml of sodium sulphite solution and kept to stand for 15 min at room temperature after mixing thoroughly. By using different concentrations of sodium sulphite and collected the precipitate by centrifugation at 3000rpm for 10 minutes. The precipitate is allowed to placed in the solution containing 20% sodium sulphite, the supernatant is removed and discarded, subsequently the precipitate is dissolved in a minimum volume of saline. Then electrophoresis carried out on the dissolved precipitate.

Organic solution precipitation[10]

All the steps carried out at 0°C. Mixing 2ml of plasma with 1ml of citrate buffer in a centrifuge tube, 7ml of ice cold ethanol is added slowly in water with stirring. Left ice for 3 minutes then centrifuged for 10 minutes at 0°c, after this process supernatant is removed and precipitate is dissolved in saline. Electrophoresis is carried out on these two solutions; the separation is compared with that obtained by salt precipitation.

Specificity of SDS-PAGE [11]

Polyacrylamide gel electrophoresis allows protein separation by differentiation migration to anode or cathode through a non reactive matrix formed by acrylamide and N,N' methylene bisacrylamide comonomers that under go free radical mediated polymerization to form a meshwork of pores

that sterically resist protein migration. Polymerization is initiated by ammonium persulfate (radical source) and catalyzed by TEMED (a free radical donor and acceptor) [Table 2]. The relative molecular weight of protein separation is determined by the local acrylamide concentration (acrylamide and bisacrylamide) and the acrylamide: bisacrylamide ratio [+].

In the absence of SDS (sodium dodecyl sulphite), the extent and direction of protein migration depends largely on the individual charge to mass ratios, and this complicates relative molecular weight estimations. SDS (an anionic detergent) partially denatures protein secondary structure and non disulphide linked oligomeric structures, conferring a net negative charge on proteins due to the highly anionic sulphite group on the detergent. Addition of reducing agent (e.g. mercaptoethanol) and heating allows complete denaturation by breaking the intra and inter disulfide bridges and by linearising the polypeptide chains by thermally overcoming folding kinetics [Table 3]. Polypeptide linearization and binding allow migration to occur solely on basis of relative molecular weight since all protein species carry net negative charges and display an anodal migration[12].

For an improved separation in SDS-PAGE, discontinuous, rather than continuous buffer system is preferred (A continuous system has only a single separating gel and uses the same buffer in the tanks and gel) [Table 4]. In a discontinuous buffer system, a non restrictive large pore gel, called a stacking gel is layered on top of a separating gel called a resolving gel. Each gel is made with a different buffer, and the tank buffers are different from gel buffers [13].

Staining of SDS-PAGE gels: The staining of SDS-PAGE gels is carried by coomassie blue staining, this method has a reported sensitivity limit around 1 Ig/band where as silver staining extends to below 200ng/band. However, coomassie staining is suitable for gels prepared for purification tablets, and if the target protein is available in large amounts. There are methods where staining and distaining times are reduced by microwaving. Although this does speed up the process, the hot methanol fumes are not pleasant. The solution required for staining, coomassie stain (0.5% m/v in 50% v/v methanol). Coomassie stain blue R-250 (0.5g) and methanol (250ml) are added to a 500 ml volumetric flask and made up to volume with distilled water. The solution is stirred until the stain is completely dissolved (30 min- 1 hour at RT). Destain solution I (50% v/v methanol in distilled water), Destain solutions II (5% v/v methanol in distilled water) **[Table-5]**. The procedure follows by disassembling the electrophoresis plates from the SDS-PAGE apparatus and rinsed briefly with distilled water [14]. The glass plates separated with a plastic spacer, the gel appears to be sticking to one of the plates. The plates and gel placed into the Tupperware and add distilled water until the plates are submerged. The plates will separate easily and the gel will pulled away, water is drained off and excess coomassie stain is added and kept on a shaker for 4 hours. After 4 hours the stain is decant back in to the bottle (it can be reused 3-5 times) and excess destain solution I poured on to the gel and shake for 4 hr. the destain I poured off and excess destain II solution is poured, shaked until completely distained and fully rehydrated. The gel can be stored in destain II until photographed or dried .

Table 1: Concentrations of different protein fractions in normal human plasma

DIFFERENT PROTEINS	CONCENTRATION
Albumin	55.2%
Globulin	44.8%
α_1 globulin	5.3%
α_2 globulin	8.7%
β globulin	13.4%
γ globulin	11.0%
Fibrinogen	6.5%

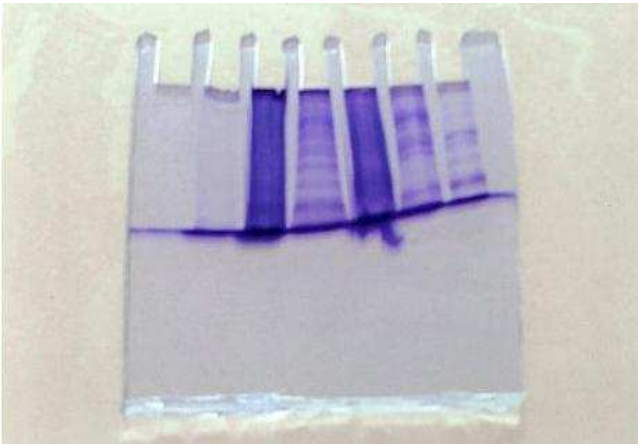

Fig 1: Different protein bands on gel electrophoresis

Table 2: For Polyacrylamide gel

1M Tris-Cl (pH 8.8)	10x Electrophoresis buffer
1 M Tris-Cl (pH 6.8)	30g Tris
20% SDS	145g Glycine
40% Acrylamide (37.5:1 acrylamide:bisAcrylamide)	10g SDS
10% Ammonium per sulfate (store aliquots@ -20°c), TEMED, 2% Agarose, 0.01%SDS	Bring 1 lit with H2O

Table 3: 6X Sample buffer (store aliquots at 20°c)

6x composition	For 10ml
60% glycerol	6.0 ml in 100% glycerol
300 Mm Tris(pH 6.8)	3.0 ml 1 M Tris 6.8
12mM EDTA	240 ul 0.5 M EDTA
12% SDS	1.2 g SDS
864 2-mM mercaptoethanol	600 ul 2-mercaptoethanol
0.05% bromo phenol blue	pinch bromophenol blue

Table 4: Separating Gel Percentage (vol in mls)

5%	6%	7%	8%	9%	10%	11%	12%	13%	14%	15%	Final conc.
3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	375mM
	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	01%
0.05	1.50	1.75	2.00	2.25	2.50	2.75	3.00	3.25	3.50	3.75	5-15%
1.25	4.70	4.45	4.20	3.95	3.70	3.45	3.20	2.95	2.70	2.45	
4.95											

Table 5: For staining/Destaining gel

Coomassie staining solution	Destaining solution
0.1% Coomassie brilliant blue R-250	40% Ethanol
40% Ethanol	10% Acetic acid
10% Acetic acid	Gel equilibrium buffer 20% ethanol 10% glycerol
(Dissolve coomassie in ethanol before adding water and acetic acid will require stirring 1-2 hours	

RESULTS AND DISCUSSION

Early stage of cancer detection is the first step toward successful clinical therapy and increased patient survival. Clinicians monitor cancer progression by profiling tumor cell proteins in the blood plasma of afflicted patients. Blood plasma, however, cancer protein assessment is a difficult because it is rich in albumins and heterogeneous protein species. We report herein a method to detect the proteins released into the circulatory system by tumor cells.

This study was conducted for qualitative analysis of serum proteins separated by SDS-PAGE and stained by Coomassie Brilliant Blue R-250 and detailed salt fractionation in order to describe the preliminary identification of serum proteins (variations in the size of the globulin fractions) that may act as diagnostic marker in benign cancer patients.

We obtained varied patterns of serum of multiple myeloma. The most characteristic finding was translation process. Over production of serum proteins like gamma and beta globulins in breast and rectal cancer is seen. The narrow bands most often found with the γ globulins. Then it is seen diminishing frequency between SDS- PAGE with low toxicity staining the γ and β- globulins with pre β and β - globulins (Fig. 1). Some of overlapping patterns indicate the presence of M protein and Bence jones proteins, K (kappa) and lambda chains of abnormal immunoglobulins. IgG, IgM, IgE, IgD, These types of proteins are also called as Para proteins [14, 15] (Fig.1). A similar intense narrow band as like seen in multiple myeloma occurs in 'macroglobulinemia' and occasionally in other diseases of the reticuloendothelial system, which may be congenital or acquired, and other globulin fractions, are unaltered.

To distinguish between normal and pathological state of benign cancer, a qualitative analysis of Coomassie Brilliant Blue-stained proteins separated by SDS-PAGE was undertaken in this study. To conclude, we report data and a method for detecting proteins released into the circulatory system by the tumor microenvironment. This pilot approach may lead to the identification of novel protein markers in blood and provide a new method of identifying tumor biomarker profiles for guiding both early detection and therapy of human cancer.

CONCLUSION

To conclude, reported data and a method for detecting proteins released into the circulatory system by the tumor microenvironment. This pilot approach may lead to the identification of novel protein markers in blood and provide a new method of identifying tumor biomarker profiles for guiding both early detection and therapy of human cancer.

REFERENCES

1. Armstrong AC. Eaton D, E wing JC. Cellular immunotherapy of cancer. *Br med J.*,2001; 323: 1289-93
2. LH.Hartwell and MB kastan. *Cell cycle control and cancer.1994;* 266
3. Anderson W F. gene therapy scores against cancer. *Nat med., 2000;*6: 862-63.
4. Frances A shepherd, Srikla S.sridhar. Angiogenisis inhibitors under study for the treatment. *Lung cancer.*2003; 41: 563-72
5. Kathleen Collins, Tylor jacks an Nikola P.pavleich. The cell cycle and cancer PNAS.1977; 34: 7
6. Kuper H, Boffetta P, Adami HO. tobacco use and cancer causation association by tumour type, *Journal of internal medicine.* 2006;252(3): 206-24
7. Sheren keleg, Peter Buchler, Roman Ludwig, Markus w buchler, Helmet frieess,. Nvasion and metastasis in pancreatic cancer. *Molecular cancer* 2010;3(1), 15-22
8. Sarah Ahmed saimanaz. Farkhanda ghafoor, M.saleem akhtar. Electrophoretic analysis of serum proteins in prostate cancer. 2004;43: 1.
9. Sumi S, Arai K,Yoshida K. separation methods applicable to prostate cancer diagnosis and monitoring therapy. *J Chromatogr B biomed Sci Appl.*,2001; 764(1-2): 445-55.
10. Klaus Weber and Mary Osborn. The reliability of molecular weight determinations by dodecyl sulfate poly acrylamide gel electrophoresis. *The journal of Biochemistry.,1969;* 244: 16 4406-12.
11. Shapiro, viffuella and maizel, *Bio chem.. Biophys. Res Commun.1967;* 815.
12. G.I. Abelev. Production of embryonal serumá-globulinby hepatomas: review of experimental and clinical data. *Cancer research*,1968 28: 1344-50.

13. Adjei AA. Blukening of oncogenic radioactive signaling for cancer therapy. *J.Natl cancer institute*. 93: 1062-1074 (2001).
14. Muller-Eberhard HJ. A new supporting medium for preparative electrophoresis. *Scand J Clin lab., Inv., 1960;*12: 33-42.
15. Hermans J.F, and Hermans. M-Th Immuno electrophoresis. *Acta med.Scand 1961;* 367: 27-34.