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SERUM ELECTROPHORESIS PATTERN IN BREAST CARCINOMA IN DIFFERENT POPULATIONS IN AN AROUND CHENNAI CITY, TAMIL NADU.

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ABSTRACT

Serum protein electrophoresis is widely used in clinical laboratories, especially for the detection and identification of paraproteins. Traditional clinical electrophoretic procedures are manual methods that use agarose gels or cellulose acetate membranes as the separation bed. Quantitation of the five major serum fractions is done by densitometric scanning of the gel or the membrane. Clinical interpretation is based on the alteration of the content of one or more of the five fractions. Agarose as the supporting medium for protein electrophoresis is reported to give better resolution than cellulose acetate, with increased ability to detect paraproteins. Densitometric scanning depicted five serum protein fractions albumin, α_1 albumin, α_2 albumin, β globulin, and γ globulin in breast cancer subjects. The present also reported that ages of the three groups differed (31-40, 41-50, 51-60 yrs), correlation analysis between serum protein levels and the age of the patients failed to reveal any statistically significant relationship. The percentage of the different serum protein fractions obtained was in the normal range like the control.

KEYWORDS: Serum protein Electrophoresis, Immunoglobulin, Densitometer scanning, Breast cancer.

INTRODUCTION

The plasma proteins form an extraordinarily complex mixture of which our knowledge has increased considerably with the development of new techniques. Most plasma proteins are

synthesized in the liver; immunoglobins are produced by the reticuloendothelial system, lymph nodes and plasma cells; and enzymes are released from various organs. The techniques used to separate the various groups of proteins from each other and to demonstrate the presence of the particular proteins in them on certain properties of the proteins. These depend mainly on the kind of electrically charged groups present in the protein molecule, which determines the type and strength of the net charge this carries, whether it is hydrophobic or hydrophilic, and the weight and shape of the protein molecule. Changes in the various fractions of serum proteins, particularly alpha glycoproteins, have been reported many times in cancer patients (Winzler and Smyth, 1948; Bacchus *et al*., 1967; and Synder and Ashwell, 1971), and although similar changes are seen in many nonmalignant conditions, for instance, inflammation, the measurement of specific glycoproteins has indicated that specific protein profiles may exist for malignant and non-malignant disease, and even for different types of malignancy (Synder and Ashwell, 1971 and Douma & Van Dalen, 1974).

MATERIALS AND METHODS

The SAS-MX serum protein kit is intended for the separation and quantitation of serum protein by agarose gel electrophoresis. Serum contains over 100 individual protein, each with set of functions which are subject to specific variation in concentration under different conditions: (i) Since the introduction of moving boundary electrophoresis by Tiselius and (ii) the subsequent use of zone electrophoresis, serum protein have been fractionated on the basis of their charge at a particular pH. The SAS-MX serum protein kit separated serum proteins into 5 main classes (albumin, alpha I- globulin, alpha 2-globulin, beta-globulin, and gamma globulin) according to the charge in an agarose gel. The proteins are then stained to allow visualization and quantitative interpretation. Each of the classical electrophoretic zones, with the exception of albumin, normally contains 2 or more components. The relative proportions of these fractions have proven to be useful aids in the diagnosis and prognosis of certain disease states.

Composition

1. SAS-MX Serum Protein Gel

Contains agarose in a Tris/barbital buffer with thiomersal and sodium azide as preservative.

2. Tris/barbital Buffer Concentrate

It contains barbital and sodium barbital with sodium azide as preservative. Before use, the contents of the bottle were diluted with 900ml of purified water and mixed well. Buffer salts

may crystallize slightly on standing. Crystals from the bottle were washed with dilute buffer to ensure complete dissolution.

3. Acid Blue Stain Concentrate

Each bottle contains concentrated Acid Blue stain, the contents of the vial were diluted in 700ml of purified water. The concentrate was stirred overnight and filtered before use. The stain was stored in a tightly closed bottle.

4. Destain Solution Concentrate

Each bottle contains 40ml of concentrated destain solution. The contents of each vial were diluted in 2 liters of purified water and stored in a tightly stoppered bottle.

5. Other Kit Components

Each kit contains instructions for use, sufficient sample application templates and blotters A and C complete 10 gels.

Procedure for electrophoresis

The gel was placed a paper towel. The gel surface was blotted with a blotter C and the blotter was discarded.The sample application template was aligned with the marks at the edge of the gel. Blotter A was placed on top of the template and rubbed with a finger across the slits to ensure good contact. The blotter A was removed and retained for use in step 5. 3µl of sample was applied to each slit and allowed to absorb for 4 minutes. While the sample was absorbing, approximately 25ml of buffer was poured into each inner section of the SAS- MX Chamber.

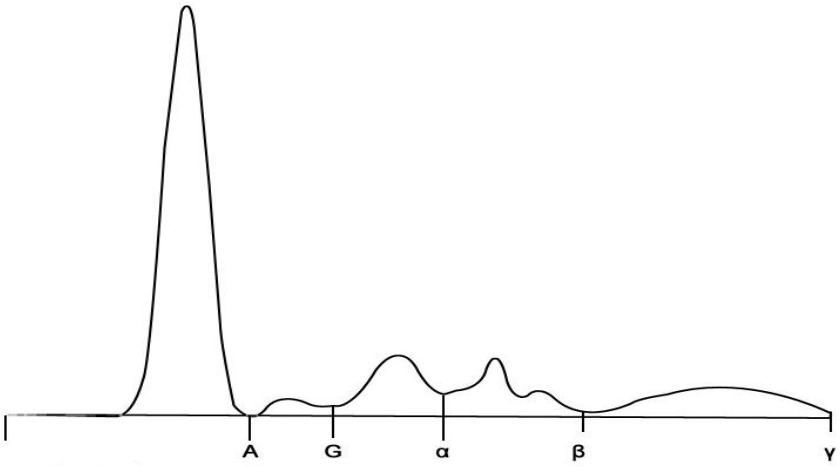
Following sample absorption, the template was lightly blotted with the Blotter A retained from step 2. Both blotter and template were removed. The gel was positioned in the chamber agarose side down aligning the positive (+) and negative (-) sides with the corresponding positions on the chamber. The gel was subjected to electrophoresis at 80 volts for 30 minutes. At the end of the electrophoresis, the gel was completely dried in an IOD or other laboratory oven with forced air at 60 - 70° (The drying of the plate should take no more than 5-10 minutes to prevent diffusion of bands. If this cannot be achieved, fix the gel for 5 minutes in methanol prior to drying). The dry gel was immersed in stain solution for 10 minutes. The gel was destained in 2 x 60 second washes of destain solution or until the background is clear.

The gel was washed briefly in purified water to remove all traces of destain and dried at 50-60°C.

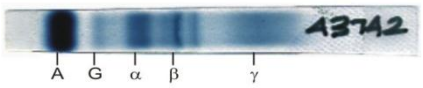
RESULTS

The fastest moving band and normally the most prominent was the albumin band found closest to the anodic edge of the plate. The faint band next to this is alpha1 globulin, followed by alpha2 globulin, beta, and gamma globulins. On electrophoretic analysis of serum protein, revealed six distinct bands in Coomosie Brilliant Blue staining on the gel. The main components of the α_1–region were α_1–lipoproteins, α_1–antitrypsin and α_1–acid glycoproteinand the α_1–antitrypsin predominated the intensity of the band. Similarly the main component of α_2–band was α_2–macroglobulin, constituting about 75% of the α_2– globulins. A faint band was seen midway between the α_2 and β regions and had been characterised by cold insoluble globulin and was due to transferrin and β_2–lipoproteins. The β_2–region was also characterised by complement factors 3 and 4 giving a band sharp at the cathodic edge but less so at the anodic site. The greater part of the immunoglobulins was in the γ region contributed to some extent, though diminishingly towards the anode, to the background colour stretching as far as the α_2 region. Densitometric scanning depicted five serum protein fractions albumin, α_1 albumin, α_2 albumin, β globulin, and γ globulin in breast cancer subjects.

Although the ages of the three groups differed (31-40, 41-50, 51-60 yrs), correlation analysis between serum protein levels and the age of the patients failed to reveal any statistically significant relationship. The percentage of the different serum protein fractions obtained was in the normal range like the control. The densitometry graph obtained (Fig, 1, 2, 3 & 4) was similar to the control. The method used was sensitive to 0.3g/L per band determined as the lowest concentration of protein, and was evident as a discrete band on the completed gel (Tables1, 2, 3 & 4).

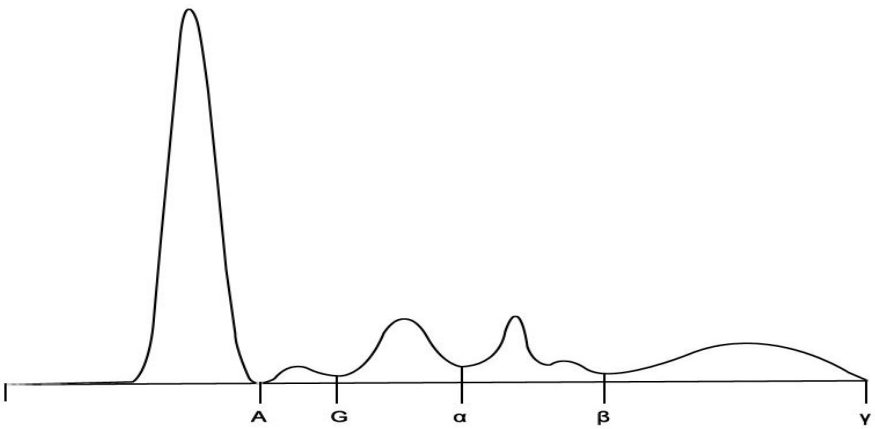


Fractions	%	Ref. %
Albumin	61.6	52.0 - 65.0
Alpha 1	2.5	2.5 - 5.0
Alpha 2	11.6	7.0 - 13.0
Beta	10.4	8.0 - 14.0
Gamma	13.9	12.0 - 22.0

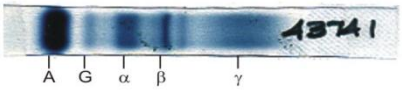


A/G Ratio : 1.6

Fig. 1: Serum Protein Electrophorogram.

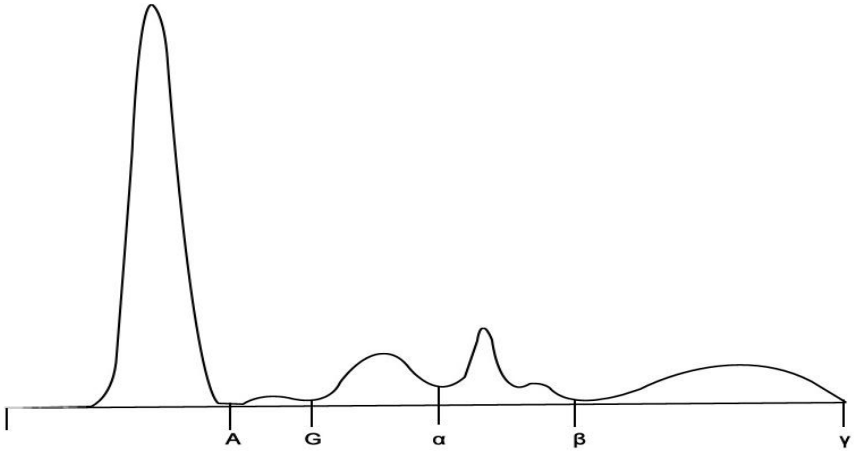


Fractions	%	Ref. %
Albumin	58.2	52.0 - 65.0
Alpha 1	2.1	2.5 - 5.0
Alpha 2	12.5	7.0 - 13.0
Beta	10.1	8.0 - 14.0
Gamma	17.1	12.0 - 22.0

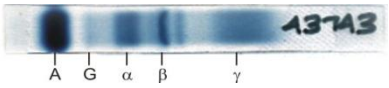


A/G Ratio : 1.39

Fig. 2: Serum Protein Electrophorogram.

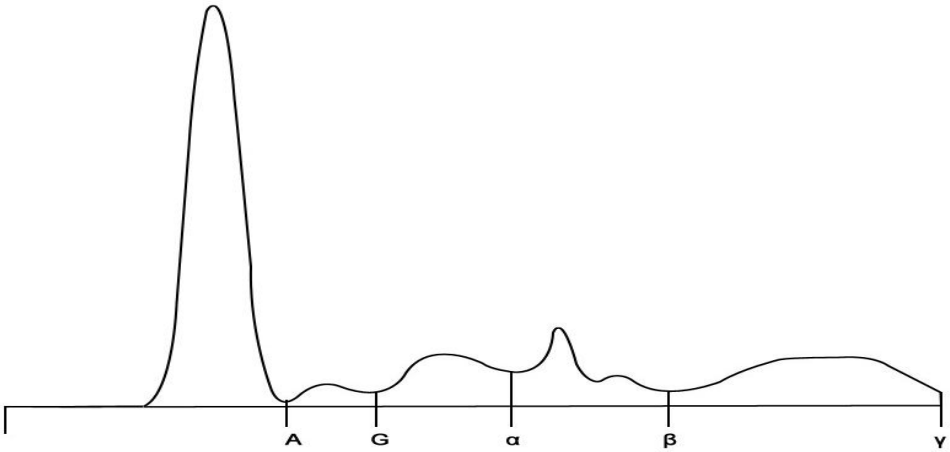


Fractions	%	Ref. %
Albumin	62.3	52.0 - 65.0
Alpha 1	1.1	2.5 - 5.0
Alpha 2	10.9	7.0 - 13.0
Beta	10.0	8.0 - 14.0
Gamma	15.7	12.0 - 22.0

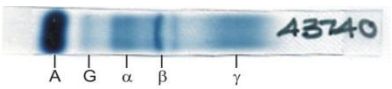


A/G Ratio : 1.65.

Fig. 3: Serum Protein Electrophorogram.



Fractions	%	Ref. %
Albumin	52.5	52.0 - 65.0
Alpha 1	3.1	2.5 - 5.0
Alpha 2	11.6	7.0 - 13.0
Beta	12.1	8.0 - 14.0
Gamma	20.7	12.0 - 22.0



A/G Ratio : 1.11

Fig. 4: Serum Protein Electrophorogram.

Table 1: Serum Protein Electrophoresis Report.

Test Description Protein Electrophoresis	Observed Value	Reference Range
Total Proteins (Biuret)	7.7	60 - 8.3 gm/dl
Albumin	58.2	52 - 65%
Alpha 1 Gobulin	2.1	25 - 50%
Alpha 2 Gobulin	12.5	7 - 13%
Beta Globulin	10.1	8 - 14%
Gamma Globulin	17.1	12 - 22%
Albumin / Globulin Ratio	1.40	

A/G Ratio : 1.39

Fractions	%	Ref. %
Albumin	58.2	5.20 - 65.0
Alpha 1	2.1	2.5 - 5.0
Alpha 2	12.5	7.0 - 13
Beta	10.1	8.0 - 14.0
Gamma	17.1	12.0 - 22.0

Table 2: Serum Protein Electrophoresis Report.

Test Description Protein Electrophoresis	Observed Value	Reference Range
Total Proteins (Biuret)	7.2	60 - 8.3 gm/dl
Albumin	61.6	52 - 65%
Alpha 1 Gobulin	2.5	2.5 - 5.0%
Alpha 2 Gobulin	11.6	7 - 13%
Beta Globulin	10.4	8 - 14%
Gamma Globulin	13.9	12 - 22%
Albumin / Globulin Ratio	1.60	

A/G Ratio : 1.6

Fractions	%	Ref. %
Albumin	61.6	52.0 - 65.0
Alpha 1	2.5	2.5 - 5.0
Alpha 2	11.6	7.0 - 13.0
Beta	10.4	8.0 - 14.0
Gamma	13.9	12.0 - 22.0

Table 3: Serum Protein Electrophoresis Report.

Test Description Protein Electrophoresis	Observed Value	Reference Range
Total Proteins (Biuret)	7.6	52 - 8.3 gm/dl
Albumin	52.5	52 - 65%
Alpha 1 Gobulin	3.1	2.5 - 5.0%
Alpha 2 Gobulin	11.6	7 - 13%
Beta Globulin	12.1	8 - 14%
Gamma Globulin	20.7	12 - 22%
Albumin / Globulin Ratio	1.10	

A/G Ratio : 1.11

Fractions	%	Ref. %
Albumin	52.5	52.0 - 65.0
Alpha 1	3.1	2.5 - 5.0
Alpha 2	11.6	7.0 - 13.0
Beta	12.1	8.0 - 14.0
Gamma	20.7	12.0 - 22.0

Table 4: Serum Protein Electrophoresis Report.

Test Description Protein Electrophoresis	Observed Value	Reference Range
Total Proteins (Biuret)	8.1	60 - 8.3 gm/dl
Albumin	62.3	52 - 65%
Alpha 1 Gobulin	1.1	2.5 - 5.0%
Alpha 2 Gobulin	10.9	7 - 13%
Beta Globulin	10.0	8 - 14%
Gamma Globulin	15.7	12 - 22%
Albumin / Globulin Ratio	1.70	

A/G Ratio : 1.65

Fractions	%	Ref. %
Albumin	62.3	52.0 - 65.0
Alpha 1	1.1	2.5 - 5.0
Alpha 2	10.9	7.0 - 13.0
Beta	10.0	8.0 - 14.0
Gamma	15.7	12.0 - 22.0

DISCUSSION

Changes in the various fractions of serum proteins, particularly α glycoproteins, have been reported many times in cancer subjects (Synder and Ashwell, 1971). Although similar changes are seen in many non-malignant conditions, (eg. Inflammation), the measurement of specific glycoproteins has indicated that specific protein profiles may exist for malignant and

non-malignant disease, and even for different types of malignancy (Douma and Van Dalen, 1974).

The main components of the plasma proteins are albumin, α_1- acid glycoprotein, α_1 – Antitrypsin, α_1– Fetoprotein, Transcortin, Thyroxine binding globulin, α_1 – Antichymotrypsin, β globulin, γ-Globulin, transferin, hemopexin, plasminogen, fibrinogen etc. The total plasma protein concentration normally ranges from 60-80g/l. Albumin ranges from 35-50 g/l and globulin from 20 to 35 g/l. The measurement of total protein concentration may be altered by changes in plasma volume; an increase is caused by dehydration and a decrease due to the excess of water content in the plasma. The present investigation showed a significant increases in total protein concentrations, which may have arisen from an increase in total globulins, usually the result of decrease in albumin level. The biochemical estimations albumin and globulin support the above proposition in the present investigation. α_1 acid glycoprotein is usually high in patients with cancer, but its general usefulness as a tumor marker has not yet been established (Gowenlock, 2002). In the present investigation, a distinct band at α_1 region indicates the rise in the α_1 acid glycoprotein. The exact function of this protein (normal value 0.3- 1.0 g/l), formerly called orosomucoid is uncertain but it is an acute phase of protein where the α_2 comprises varying glycoproteins such as Zn-α_2 glycoprotein, α_2 -HS glycoprotein etc. In the present investigation, a distinct band in this region confirms a rise in the α_2 glycoproteins. The biological functions of many of these glycoproteins are numerous and not all are known, neither are the mechanisms, by which changes are induced by cancer. It is possible that glycoproteins are synthesized directly by the tumor, but in view of the diversity of protein changes, it seems more likely that the malignant process is indirectly affecting the metabolism of these proteins (Pettingle and Tee, 1977). The other components of α_2 zone are α_2 macroglobulin, haptoglobulins and ceruloplasmin (in small amounts). α_2 macro globulin has been reported to increase during pregnancy, cirrhosis, malignancy and diabetes (Gowenlock, 2002). Raised serum ceruloplasmin has been reported previously in patients with both cancer and chronic inflammatory diseases. (Synder and Ashwell 1971). The occurrence of a bright band at α_2 zone in the present study prompt to presuppose that there might be a rise in α_2 macroglobulin and ceruloplasmin due to malignancy.

Unfortunately, in spite of a considerable number of serum protein surveys performed in the subject of carcinoma, many of the reports are not comparable or it conflicts with each other. Many studies have not been confined to a single type of cancer nor taken account of the extent of the disease; the nature, age and state of health of the control subjects. There appeared to be differences in the serum protein levels in women with breast cancer including the benign condition. These differences are seen preoperatively at the earliest time of clinical detection of a breast tumor and do not appear to be related to the age difference between the groups. (Pettingle and Tee, 1977). But an increase in β glycoprotein levels does not appear to have been reported before. In previous studies the levels have been either unchanged (Cleve, 1968) or reduced (Synder and Ashwell, 1971). The plasma concentration of a protein is dependent on synthesis, degradation and loss, therefore the change in the individual proteins will be different. Protein synthesis is switched to the protective proteins at the expense of transport proteins therefore there is a concomitant fall in albumin and transferrin. (Gowenlock, 2002).

Our study, however, is confined to one type of cancer at an early stage and is not strictly comparable with the previous reports. Densitometric scanning was performed to study any serum protein level difference of five principle protein fractions such as albumin, α_1 albumin, α_2 albumin, β globulin, γ globulin and it was observed that there is no significance difference in the serum proteins of the patients when compared with the control. Though biochemical estimation of serum albumin in the breast carcinoma subjects showed a decrease in the concentrations, the fall in the albumin concentration is not so high to be evident in the discrete band formation during electrophoresis. Quantitation of proteins using densitometry will not show major differences if at all there is a major decrease or increase in the concentration. So, the normal graphical interpretation given for the serum electrophoresis of proteins of breast cancer subjects using densitometry is not surprising (Gowenlock, 2002).

Possible nutrition alterations that is decreased albumin and protein was explained in many types of cancer (Winningham *et al.,* 1994; Nail and Jones, 1995 and Brophy and Sharp, 1991). The present study shows that there are not much significant changes in the serum protein fraction distribution in the subjects of earlier stage of disease except for the glycoprotein at the α_1 and α_2 zones.

It is possible that glycoproteins are synthesized directly by the tumor, but in view of the diversity of protein changes, it seems more likely that the malignant process is indirectly affecting the metabolism of these proteins. Their synthesis in the liver is known to be influenced by many hormones (Hoch-Ligeti & Good *et al.*, 1971) and hence the relationship of any hormone and protein changes in the breast cancer patients has to be investigated for a longer period with prospective follow-up of the patients, which needs several years in order to establish the value of any prognostic profile of serum protein changes and its relationship to the pathogenesis.

CONCLUSION

The greater part of the immunoglobulins was in the γ region contributed to some extent, though diminishingly towards the anode, to the background colour stretching as far as the α_2 region. Densitometric scanning depicted five serum protein fractions albumin, α_1 albumin, α_2 albumin, β globulin, and γ globulin in breast cancer subjects. The present also reported that ages of the three groups differed (31-40, 41-50, 51-60 yrs), correlation analysis between serum protein levels and the age of the patients failed to reveal any statistically significant relationship. The percentage of the different serum protein fractions obtained was in the normal range like the control.

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