Paper 2

Patterns of human serum proteins in malignant cases
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The application of electrophoretic techniques gives a specific pattern for the different types of proteins present in the human serum, through their separation according to their relative migration rates (Tarnoky, 1968). The position, width and intensity of these bands are characteristic for normal sera and a particular protein can be identified according to these criteria from the entire pattern. Attempts have been made to utilize these characteristics in identifying variations in proteins associated with different diseases.

Variations in the serum protein have been observed in different malignant growths and have been suggested to be useful in their diagnosis (Rohlfing et al., 1969a, b; Werthamer and Amaral, 1971, 1976). A significant impetus was given in the search for specific diagnostic methods in detecting cancer, following the demonstration of alpha₁-foeto protein (Tatarinov, 1966; Abelev et al., 1967; Abelev, 1968; Alpert et al., 1968; Sankale et al., 1968; Bourrielle et al., 1970; Geoffrey et al., 1970), carcinoembryonic antigen (Gold and Freedman, 1965; Thomson et al., 1969) and EBV related antigens. A simple specific test of the patient's blood has not, however, yet been developed. Other methods used include different types of electrophoresis, amongst which the technique of disc acrylamide gel electrophoresis, developed by Ornstein (1964).
and Davis (1964) gives an excellent resolution of the serum proteins. More than twentyfive distinct protein bands can be identified through this method in the normal human serum. To some extent this pattern varies with age and with polymorphisms of genetically determined protein variants.

When applied to sera from different forms of cancer, certain authors were able to obtain characteristic patterns on disc acrylamide gels with chronic lymphatic leukaemia (Werthamer and Amaral, 1971). The same authors claimed the presence of a consistent and distinctive pattern in the gel for the different cancers (Werthamer and Amaral, 1976).

Variable protein patterns corresponding to different cancer types have been observed by a number of other workers, as for example,

Bronchial cancer (Adamczyk and Czechowicz, 1969)
Breast cancer (Rosato, 1967; Guliaeva, 1969)
Burkitt's lymphoma (Klein et al., 1966; Old et al., 1966)
Cervical cancer (Michalica et al., 1972)
Chronic lymphocytic leukaemia (Geiro, 1966; Amaral, 1966a; Werthamer and Amaral, 1971, 1976)
Colonic carcinoma (Gold and Freedman, 1965)
Gastric cancer (Oszaski et al., 1967; Kurtenkov, 1970; Konishi et al., 1974)
Malignant gynecological tumours (Dvorak et al., 1968)
Haematologic neoplasms (Rohlfing et al., 1969b)
Hepatomas (Tatarinov, 1966; Alpert et al., 1968; Uriel and Nechand, 1968; Burtin, 1969; Foli et al., 1969; Iammarino, 1969; Lippi et al., 1969, 1971; Nechand et al., 1969; Canrot et al., 1970; Portugal et al., 1970; Nishioha et al., 1971)
Hodgkin's disease (Sega et al., 1971, 1973a, b)
Hypernephroma (Bichler et al., 1974)
Laryngeal cancer (Brovko and Kizam, 1966; Bodea et al., 1969; Skonieczny, 1973)
Leukaemia (Starkiewiczowa et al., 1963; Higazi et al., 1966; Perolini et al., 1966; Castuma et al., 1968; Nomura, 1968; Raszek et al., 1969; Spina et al., 1970)
Lung cancer (Bonchek, 1971)
Lymphoma (Castuma et al., 1968; Rohlfing et al., 1969a)
Lymphomyeloid malignancies (Gupta et al., 1974)
Malignant tumours (Talerman and Haije, 1973; Adamczyk, 1976)
Melanoma, sarcoma, carcinoma and cancer of the haematopoietic tissues (Hughes, 1971, 1972)
Myeloma (Kochwa et al., 1964; Dammacco and Pinto, 1967; Kunkel, 1968; Saraebasi et al., 1968; Amaral, 1969b; Fishkin et al., 1970)
Myoma (Roszkowski et al., 1968)
Prostatic cancer (Ablin et al., 1971, 1973; Dhar et al., 1973)
Stomach cancer (Akai et al., 1965; Oszacki et al., 1967;
Woszczyk and Reslinski, 1969; Bourreille et al.,
1970; Geoffrey et al., 1970; Kurtenkov, 1970;
Podil'chak, 1972; Szczepaniak, 1972; Konishi
et al., 1974)
Cancer of the digestive tract (Suszczeewska-Fischerowa, 1963;
Thomson et al., 1969)
Cancer of the female genital system (Razzini and Gatta, 1965)
Cancer of the uterus (Roszkowski et al., 1968; Madajewicz et al.
1971)
Cancers of different origin (Berndt, 1963; Granan, 1963; Kato,
1966; Surodeikina, 1966; Abelev et al., 1967;
Masopint et al., 1968; Scholz et al., 1968;
Uriel et al., 1968; Strizhova, 1969; Turief
et al., 1970; Sega et al., 1971; Maisin et al.,
1972; Werthamer et al., 1973; Ewtiukhin, 1975;
Baksi et al., 1976)

Maisin et al. (1972) observed a significant difference
in the protein pattern in sera obtained from cancer patients
and those from normal individuals, especially when the sera
were incubated prior to electrophoresis with total yeast RNA.
The observations of Werthamer and Amaral (1976) and of Hellstrom et al. (1969) and Perlmann et al. (1973) show that the immunosuppressive proteins, some of which were caused by malignant conditions, may be present in the sera of cancer patients. They have, therefore, recommended the use of disc polyacrylamide gel electrophoresis to detect patterns that are consistently different from the normal or may indicate change in quantity of normal proteins. They observed that the sera from patients with carcinoma and certain lymphomas produce distinctive electrophoretic patterns. Reports from other laboratories also show the occurrence of fairly specific protein bands which can be correlated with the type of cancer (Abelev, 1968; Tarnoky, 1968; Fishkin et al., 1970; Lippi et al., 1971; Werthamer and Amaral, 1971, 1976; Amaral and Werthamer, 1974).

The present work was undertaken on sera obtained from individuals with different forms of cancer, in order to study their total protein patterns through disc acrylamide gel electrophoresis, and to correlate them, if possible, with the type of cancer. A control was maintained with sera obtained from normal individuals for comparison.
MATERIALS AND METHODS

MATERIALS:

Blood Samples: Blood was collected from patients with different forms of malignancy at the Institute of Post Graduate Medical Education and Research in Calcutta. For normal control, blood was obtained from healthy donors at the local blood bank. All the samples were allowed to clot and the separated sera were stored at -10°C, till they were analysed. Sera from a total of 102 patients suffering from different types of cancer were analysed.

Reagents: All stock solutions were prepared from freshly distilled deionised water. It was essential to keep out air from the final gel mixture, because of its two-fold adverse effect. Primarily air inhibits polymerization and secondarily bubbles formed during the gelling process may be trapped within the gel. All the solutions were stored in amber coloured bottles in cold.

Solution A: 22.5 g of Acrylamide CH$_2$=CH$_2$CONH$_2$ and 0.6 g of N,N'-Methylene bisacrylamide (CH$_2$:CH$_2$:CO,NH) =CH$_2$ (BIS) were dissolved and made upto 100 ml with deionised distilled water. The amounts of acrylamide which is the monomer and bisacrylamide which serves as the co-monomer determine the final gel concentration, which is 7.5%.
Solution B: 17.15 g of Tris (hydroxymethyl)-amino methane \( \text{C}_4\text{H}_{11}\text{NO}_3 \) (Tris), 0.4 ml of \( \text{N}_3\text{N}_3\text{N}_3\text{N}_3'\) Tetramethyl-ethylenediamine \( (\text{CH}_3)_2\text{N}^+\text{CH}_2\text{N}^-\text{CH}_2\text{N}^+\text{CH}_3 \) (Temed) and 24.0 ml of Normal Hydrochloric acid \( 1(\text{N}) \text{HCl} \) were dissolved and made up to 100 ml with deionised distilled water. This solution is the gel buffer and has a pH of 8.6.

Catalyst: Ammonium persulphate, which serves the purpose of a catalyst was prepared daily by dissolving 30 mg of the salt \( (\text{NH}_4)_2\text{S}_2\text{O}_8 \) in 10 ml distilled water.

2M Sucrose: It was prepared by dissolving 6.84 g Sucrose \( \text{C}_{12}\text{H}_{22}\text{O}_{11} \) in 10 ml distilled water.

Indicator: The indicator, bromophenol blue was prepared by dissolving 0.1 g of the salt in 10 ml 2M Sucrose.

Bridge Buffer: 0.6 g Tris (hydroxymethyl)-amino methane \( \text{C}_4\text{H}_{11}\text{NO}_3 \) (Tris) and 2.88 g Glycine \( \text{H}_2\text{N}^+\text{CH}_2\text{COOH} \) were dissolved and made up to 100 ml with deionised distilled water. This solution is the reservoir buffer and was diluted ten times before use. It has a pH of 8.3.

Stain (Amido Black solution): 0.1 g of Amido Black or Naphthalene black was dissolved in 100 ml of 7% Acetic acid.

Destaining solution: The destaining solution was freshly prepared before use. 7% Acetic acid was prepared by adding
7 ml of Glacial acetic acid to 93 ml distilled water.

**Electrophoresis apparatus:** The electrophoresis apparatus was made up entirely of Perspex, except for the platinum electrodes. The lower electrode chamber held about 300 ml of buffer, whilst the upper chamber was punched and silicone rubber grommets were inserted to hold the gel tubes. Unused holes were closed with siliconised rubber stoppers.

**Power Pack:** A constant voltage electrophoresis power supply unit manufactured by Biochem Industries was used as the source of the power supply.

**Gel Tubes:** The gel tubes were made of glass, 12 cm in length and 0.5 cm internal diameter. Scratches were made 2 cm from one end and 2.5 cm from the other end. All glass tubes were permanently siliconised and after use were thoroughly washed with detergent, and kept overnight in potassium dichromate - sulphuric acid mixture. They were then washed in running water for about 5-6 hours, followed by keeping in distilled water for the same period. The tubes were finally dried in an incubator (temperature 37°C).

**METHOD:**

A modification of the technique of vertical polyacrylamide gel electrophoresis, originally described by Ornstein (1964) and Davis (1964) was adopted for assessing the total
protein pattern.

Equal volumes of Solution A, Solution B and Ammonium persulphate solution (i.e., 10 ml each) were taken and mixed thoroughly in a test tube. The resulting gel mixture was quickly pipetted into thoroughly washed gel tubes (12 cm x 0.5 cm) securely fitted with rubber caps at one end, unto a distance of 10 cm. A drop of water was carefully added to the gel surface to level the meniscus, taking great care that it does not penetrate deep into the gel mixture. The tubes were allowed to stand for 30 minutes during which time polymerization took place. Polymerization of the gel was brought about by the action of both the catalyst - ammonium persulphate and the initiator - Temed. While the initiator was prepared as part of the gel buffer, the catalyst was kept separate until required, because of its instability. After half an hour, the lower rubber caps were removed by holding the tubes at an angle and slowly turning the caps as they were pulled down and off. One edge of the cap was first allowed to come off in order to release any pressure which would detach the gel from the sides of the tube. The overlying drop of water was shaken off and the tubes were arranged vertically in the electrophoresis apparatus.

The lower electrode chamber was filled with Tris Glycine buffer, pH 8.3 and the gel tubes were carefully inserted through
the siliconised rubber grommets of the upper electrode chamber slipping through the corresponding holes of the lower electrode chamber into the buffer solution. Ten tubes were usually handled at a time.

A drop of bromophenol blue indicator in 2M Sucrose solution was added. 20 μl of the serum was then added, followed by a drop of 2M Sucrose solution. Tris Glycine buffer, pH 8.3 was then pipetted into each of the gel tubes, to fill each tube. The upper electrode chamber was also filled with the bridge buffer submerging the upper ends of the gel tubes. At the beginning of the experiment, a current of about 1-2 mA per tube was used in order to avoid overheating and diffusion. Within a short time the bromophenol blue entered the gel and the current was raised to 4 mA per tube for the duration of the experiment (1½ hours), when the bromophenol blue band migrated up to 2.5 cm from the lower end. The tubes were never allowed to feel more than just warm to the touch as this would indicate excessive heating of the gel. All operations were carried out at 25°C.

After electrophoresis was over, the gel tubes were removed from the apparatus and the buffer shaken out. The gels were then carefully removed from the tubes with a syringe, inserted between the gel and the tube wall, with the tube held at an angle.
They were subsequently stained with 0.1% amido black or naphthalene black in 7% acetic acid in water for ten minutes, followed by destaining in the same solution with the dye. Protein bands appeared as dark blue bands on a faintly stained background. These bands were drawn on a graph paper.
OBSERVATIONS

The observations have been summarized in diagrams 1-3. The 102 cases studied have been classified under ten groups (Table I), according to the region of origin of the cancer. Both early and late stages are present. In general, the protein bands obtained after acrylamide gel electrophoresis form four distinct zones from the point of origin, which have been designated for convenience, arbitrarily as A, B, C and D.

Zone A is a distinctive feature of the cancer cases, showing a wide region of diffusely stained protein with a variable number of more intensely stained narrow bands. The zone varies in width, extending from close to the point of origin to about 35-40 position using the 100 mm notation scale (see Smith, 1968), approximately in the position of the haptoglobin (Hp) bands.

Zone B is a comparatively narrow area, occurring below zone A at about 40-45 region. It shows comparatively low stainability and may correspond to the X band of Werthamer and Amaral (1976).

Zone C is located in the middle of the spectrum at the 50 position, in the same area as occupied by transferrin (Tf). It may be single, when it is intensely stained or double, with one intense and the other faint bands. A Y-band has been
described previously in some carcinomas.

Zone D is the most fast moving region located at about the position occupied by the group specific proteins (Gc). It is faintly stained, comparatively narrow and varies in mobility. It may correspond to the transcortin (Tc) band (Werthamer et al., 1973).

The different groups of cancer present different patterns of these zones.

Group I includes 46 patients with cancers of the larynx (21 cases), pharynx (7 cases), tonsil (3 cases), laryngo pharynx (6 cases) and pyriform fossa (9 cases).

The protein pattern in twentyone cases of laryngeal cancer is rather similar with a strongly marked band at the 50 region (zone C) and a fainter band (zone B) above it, slightly above the $\beta$-glycoprotein zone. This zone is absent in one individual. There is a constant band in the alpha region, above the albumin, close to the location of the Gc group specific components (zone D). It is narrower in two cases than in the others. Zone A has a large block of diffusely stained bands ranging between about 10-15 to about 35-40; it differs amongst the individuals.

Seven cases of cancerous pharynx and three of cancerous tonsil show zone C similar to the earlier ones. Zone B differs
both in width and position from the larynx cases, as well as from each other. Zone D is more or less similar in all cases, being wider than, and located at a slightly lower level than the earlier ones. Zone A in the \( \alpha \)-position varies between the cases and a few of the bands show a more intense stain than the others.

Nine samples of cancer of pyriform fossa have zone D in more or less the same position as cancer of pharynx, but more variable in width. Zone C has the same position and intensity as in the other cases, but shows another less intense band of the same width, just below it forming a double one. Zone B is at the same level as that of cancer of larynx and zone A is variable, both in location and size, some bands showing greater intensity of staining.

In the six cases of cancerous laryngo pharynx observed, the variability is greater. Zone C shows two zones, one more intense than the other, as observed in that for cancer of pyriform fossa. In a single case, zone B corresponds in position with those of the majority of pyriform fossa cases. In the remaining five, however, they are constant and occupy a position slightly higher. Zone D is wide and occurs in more or less similar position in all the cases. Zone A is very wide in four cases and relatively narrow in two, with narrow bands having more intense stain.
In Group II, two cases of the cancer of the lung present an almost uniform pattern differing from Group I in the complete absence of zone B. Zone D is of the same width and position in both cases and zone C has two regions of intensity as the laryngo pharynx cases. Zone A does not show any distinctive features.

Group III includes ten cases of breast cancer. All the zones are present. Zone A starts at about the same level from the point of origin in all individuals, but differs in the distance of migration and the number and location of the narrow intensely stained bands. Zone B is narrow and single. Zone C has two regions of intensity, but while the upper more intense zone is constant in width and identical with earlier samples, the lower less intense zone is as wide as the upper one in all but one case. Zone D occupies a position similar to that of Group I.

Group IV including one case of salivary gland cancer and six of tongue cancer presents a rather heterogenous appearance in the width and mobility of almost all the zones. In the cancer of salivary gland, zone C is a single intensely stained region, zone B is wider and occupies a position close to the origin, zone D is narrow and located further away from the albumin than earlier cases. Zone A is closer to the origin less wide and with very few intense bands.
In the six cases of cancerous tongue, zone C is double with two regions of intensity. Zone D resembles that of salivary gland cancer in two cases, but is variable in migration pattern and intensity in the others. Zone A is rather wide, starting very nearly from the point of origin and migrating very close to zone B, which is similar to that of salivary gland cancer in five cases, but is separated into two bands in the sixth.

The first three cases of Group V suffering from Hodgkin's disease, present a uniform pattern characterized by narrow zones B and D, a broad zone A, slightly away from the origin of homogenous nature, with very few distinct bands. Zone C appears as a single wide band of medium intensity, as wide as the two bands of differing intensity, observed earlier combined.

The patterns of the two cases of lymphocytic lymphoma studied differ from those of Hodgkin's disease in having slower moving zones B and D and a narrow zone of protein in the \( \kappa \)-region (zone A). The C bands are identical.

Group VI with three cases of chronic myeloid leukaemia, presents a characteristic set up with an undifferentiated zone A and a double zone C where the more intense region is much wider than the less intense one. Zone B is variable and
narrow and approximately in the same position as in Group V, and zone D is very slow moving.

Sixteen cases of cancer of cervix, under Group VII show a wide, medium stained zone C similar to Group V and a comparatively slow D band. Zone B is absent. Zone A is faster moving than in the other groups, and a few narrow bands of greater intensity may be seen.

The two cases of hypernephroma, under Group VIII, resemble Group VI with respect to zones B and C. Zone D has a fast moving band, occupying the same position as that of Group V, and zone A is narrow and undifferentiated.

The pattern in Group IX with three cases of oesophageal cancer shows a double zone C, a few narrow bands within the undifferentiated zone A and zone B very close to zone A. Zone D is in the same position as Group VIII.

In the last group, Group X, different types of cancer have been included, such as of leg (3 cases), right arm (1 case), rectum (1 case), skin (1 case), seminova testis (1 case) and thyroid (1 case). Zone C is a single intensely stained band in the cancers of leg, skin and seminova testis, while it is double in the cancers of right arm, rectum and thyroid. Zone D is absent in the cancer of the thyroid. It
is a wide zone in the cancers of the right arm, leg and seminova testis, and narrow in the cancer of the skin, though of varying mobility in all cases. Zone B is absent in the cancers of right arm and rectum. It is narrow and of similar mobility in the other cases, except in skin cancer, where it is a fast moving band. Zone A is a narrow region with a few intensely stained bands in all cases with the exception of cancer of right arm and rectum, where it is rather wide.
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Explanation of diagram

Diagram 1 - Protein patterns of human serum following Polyscrvlemide gel Electrophoresis - Group I.
Protein patterns of human serum following Polyacrylamide gel Electrophoresis

NORMAL

CANCER TYPES

GROUP I
Explanations of diagram

Diagram 2 - Protein patterns of human serum following Polyacrylamide gel Electrophoresis - Groups II - VI.
Diagram 2

Protein patterns of human serum following Polyacrylamide gel Electrophoresis

NORMAL

CANCER TYPES

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Note: The diagram shows protein patterns for different cancer types and normal conditions.
Explanation of diagram

Diagram 3. - Protein patterns of human serum following Polyacrylamide gel Electrophoresis - Groups VII - X.
Diagram 3

Protein patterns of human serum following Polyacrylamide gel Electrophoresis

NORMAL          CANCER TYPES

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CANCER TYPES

- Group XV
- Group X
- Seminova testis
Use of polyacrylamide gel electrophoresis in the study of human serum protein patterns of malignant cases
Use of Polyacrylamide Gel Electrophoresis in the Study of Human Serum Protein Patterns of Malignant Cases

(MISS) RACHEL THOMAS*, (MRS.) ARCHANA SHARMA**
(MRS.) GEETA TALUKDER***, D. K. BHATTACHARYYA****
USE OF POLYACRYLAMIDE GEL ELECTROPHORESIS IN THE STUDY OF HUMAN SERUM PROTEIN PATTERNS OF MALIGNANT CASES

Subsequent to the development of the technique of disc acrylamide electrophoresis of serum by Ornstein (1964) and Davis (1964), attempts have been made to correlate specific alterations in the protein bands with specific diseases. The normal pattern of serum proteins itself varies with age and with the genetically determined protein variants. This method has not been yet employed for conclusive clinical diagnosis due to the large number of individual bands present and the difficulty in quantitating them according to known densitometric procedures. Some researches have been carried out on the patterns observed in malignant diseases. The different forms show different patterns, of which a few are fairly characteristic (Tarnoky, 1968; Fishkin et al., 1970; Werthamer and Amaral, 1971; Lippi et al., 1971). The present article deals with an analysis of the serum protein patterns in 68 cases of cancer following acrylamide gel electrophoresis.

MATERIAL AND METHOD

Samples were collected from cancer patients being treated at the S.S.K.M. Hospital, Calcutta. For normal control, blood was obtained from healthy donors at the local blood bank and stored at 20°C. Each sample was centrifuged and filtered and subjected to disc polyacrylamide gel electrophoresis following the method of Ornstein (loc. cit.) and Davis (loc. cit.). The gel composition was 7.5 per cent, the buffer used was Tris glycine, pH 8.3. The run was given at 4 mA per column for 90 minutes. The gels were subsequently stained in 0.1 per cent amido black or naphthalene black in 7 per cent acetic acid in water (Smith, 1968).

OBSERVATIONS

The observations have been summarised in Fig. 1. The 68 cases studied have been classified under 10 groups, according to the origin of the cancer. Both early and late stages are present. In general, the protein bands, obtained after acrylamide gel electrophoresis, form four zones from the point of origin, which have been designated for convenience, arbitrarily, as A, B, C and D.

Zone A is a distinctive feature of the cancer cases, forming a wide region of diffusely stained protein, with a variable number of more intensely stained narrow bands. The zone varies in width extending from close to the point of origin to about 35-40 position using the 0-100 mm. notation (Smith, loc. cit.), approximately in the position of haptoglobin (Hp) bands.

Zone B is a comparatively narrow band occurring below zone A, at about 40-45 region. It shows comparatively low stainability.

Zone C is located in the middle of the spectrum, at 50.0 position, in the same area as occupied by transferrin (TR). It may be single, when it is intensely stained, or double with one intense and the other faint, zones.

Zone D is the most fast moving region, located at about the position occupied by group specific components (Gc). It is faintly stained, comparatively narrow and varies in mobility.

The different groups of cancer present different patterns of these zones.

Group I, including 32 patients with cancers of larynx, pharynx, pyriform fossa, laryngo-pharynx and tonsil, shows a rather diffuse zone A, variable in size and with little differentiation into more intense bands. Zone B is single.
narrow, of more or less similar migration rate. Zone C consists, in cancers of larynx and pharynx of a single intensely stained band, accompanied in most of the other cases, by a fainter band of the same width. Zone D occupies a position between 70-75 and is narrower in the cases with cancer of larynx. All the cases show uniformity of bands irrespective of the stage of the disease.

In group II, 2 cases of cancer of lung present an almost uniform pattern, differing from the group I in the complete absence of zone B and a lower faint band under C.

Eight cases of breast cancer under group III show all four zones. Zone A starts from about 10-12. Zone C is double with the lower fainter band varying in width.

Group IV, consisting of 5 cases of salivary gland and tongue cancer, presents a rather heterogeneous appearance in the width and mobility of almost all zones. C is single in 1 case and B is double in another. Narrow distinct bands appear in zone A in salivary gland cancers.

Group V consists of 3 cases of Hodgkin's disease and 2 of lymphocytic lymphoma. In all cases zone C is wider than the groups described earlier but the staining is not intense. B and D have migrated faster in the first 3 cases and zone A occupies a larger area, starting from 5-6 position from the origin.

Group VI, with 3 cases of chronic myeloid leukaemia, is rather homogenous with undifferentiated zone A, double zone C and slow-moving zone D; B is variable.

Group VII, with 4 cases of cancer of cervix, shows comparatively fast moving zone A, little or no zone B, wide zone C and moderately fast D bands.

The 2 cases of hypernephroma under group VIII have comparatively narrow undifferentiated A and intensely stained wide C to distinguish them from the other groups.

The pattern in group IX, with 2 cases of cancer of oesophagus, shows a single intense C, a few narrow bands within the undifferentiated A zone and B very close to A.

In the last group X, different types of cancer have been included, such as, of leg (3 cases), right arm (1 case) and rectum (1 case). The C zone is single in the first 3 and double in the others.

**DISCUSSION**

Acrylamide gel electrophoresis presents a
much greater resolution of serum proteins than the other systems available. However, this property renders the identification of variants more difficult due to the presence of too many bands. On comparing the patterns present in malignancies with the normal ones, a change is recorded with advance of the disease in some cases. Characteristic patterns have been claimed for haematological disorders (Rohlfing et al., 1969), Hodgkin's disease, chronic lymphatic leukaemia and lymphosarcoma (Werthamer and Amaral, loc. cit.), amongst others listed by Tarnoky (loc. cit.). The observations made in the present article show in all cases the presence of a distinct band in zone C, corresponding to the main β-globulin band, which is wider or double in particular types. The wide diffuse staining for protein in zone A ranges from almost the point of origin to almost 40-45, with or without differentiation into positive bands in the different forms of cancer. Zone B, located above main β-globulin, is variable, both in incidence and position. The protein band in zone D occupies different locations at about the position of the group specific proteins (Gc) which are more or less similar in the cancers of the same group.

The patterns are fairly characteristic within a group but must be further categorised before they can be employed in the identification of a particular type of malignancy and in the location of the site of the primary neoplasm where only the secondary sites are known. A major handicap is the separation of genetically determined protein variants, like haptoglobin, transferrin and group specific proteins (Gc) by the method of acrylamide gel electrophoresis. The variable bands given by these genetic variants further complicate the overall picture. However, as far as the present data are concerned, in a previous communication (Baksi et al., 1976) on normal and malignant populations from the same region. it was observed that there was no relationship between the haptoglobin patterns and the incidence of malignancy. A similar study is in progress on transferrin inheritance. As most of the cases were of fairly advanced carcinoma, the variation from the normal may be marked and the intensity of staining also could be high.

Further work has been undertaken on a comparison of the protein patterns of normal members of the family with that of a patient suffering from a particular type of cancer as also the change of position and intensity of the bands after a course of radiotherapy.

SUMMARY

The serum protein patterns have been studied in 68 cases of malignant disease by polyacrylamide gel electrophoresis. The patients with nasopharyngeal, laryngeal, oesophageal and other carcinomas have been observed prior to therapy. Variations in number, position and intensity of the bands have been recorded and their main types arbitrarily classified. Particular varieties of carcinomas are found to give quite similar patterns which might be utilised in their diagnosis and prognosis.

ACKNOWLEDGMENT

The authors are grateful to Professor A. K. Sharma, Head of the Department of Botany, University of Calcutta and Dr. K. P. Sengupta, Director, Department of Pathology, IPGME & R, Calcutta for encouragement and facilities and to the University Grants Commission, New Delhi for the award of a Junior Research Fellowship to one (R.T.) of us.

REFERENCES

Paper 2B

Electrophoretic patterns of human serum proteins in malignant cases
ELECTROPHORETIC PATTERNS OF HUMAN SERUM PROTEINS IN MALIGNANT CASES

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Cyto genetics Laboratory, Department of Botany, University of Calcutta 700 019

GEETA TALUKDER
Department of Pathology, Institute for Post-Graduate Medical Education and Research, Calcutta 700 020
and
D. K. BHATTACHARYYA
Department of Pathology, North Bengal University, Siliguri.

Summary

The serum protein patterns have been studied in 34 cases of malignant disease by polyacrylamide gel electrophoresis. The patients with nasopharyngeal, laryngeal, oesophageal and other carcinomas have been observed prior to therapy. Variations in number, position and intensity of the bands have been recorded and their main types arbitrarily classified. Particular varieties of carcinomas are found to give quite similar patterns which might be utilised in their diagnosis and prognosis.

Introduction

The total protein patterns presented in the human serum after electrophoresis present certain variable features depending on age and genetic composition. A very large amount of work has been carried out on the inheritance pattern of the different protein components (Harris, 1975). Electrophoresis has been an important tool in the identification of such genetic variants (Sharma et al. 1976). Attempts have been made from time to time to correlate such patterns with individual diseases, including cancer. In a previous communication the authors had studied the total protein in a number of malignant cases and had observed certain co-relations (Thomas et al., 1976). The present article is a continuation of the work.

Materials and Methods

Samples were collected from cancer patients being treated at the SSKM Hospital, Calcutta. For normal control, blood was obtained from healthy donors at the local blood bank and stored at minus 20°C. Each sample was centrifuged and subjected to vertical polyacrylamide gel electrophoresis, following the method of Ornstein (1964) and Davis (1964). The gel composition was 7.5%; the buffer used was Tris-Glycine, pH 8.3; the run was given at a constant current of 4 mA per column for 90 minutes. The gels were subsequently stained with 0.1% Amido black or Naphthalene black in 7% acetic acid in water, followed by destaining in 7% acetic acid.
Observations

In continuation of an earlier publication (Thomas et al., 1976), 34 additional cases of cancer patients were studied. They were classified into six groups, according to the classification followed for the earlier cases in which the position and extent of each band were considered. In general, the protein bands obtained after polyacrylamide gel electrophoresis form four zones from the point of origin, which have been designated for convenience, arbitrarily as A, B, C and D.

Zone A is a distinctive feature of the cancer cases forming a wide region of diffusely stained proteins with a variable number of more intensely stained narrow bands. The zone varies in width, extending from close to the point of origin to about 35-40 position, using the 100 mm notation scale (Smith, 1968), approximately in the region of the haptoglobin (Hp) bands.

Zone B is a comparatively narrow zone, occurring below zone A at about 40-45 region showing low stainability.

Zone C is located in the middle of the spectrum at the 50 position in the same area as occupied by transferrin. It may be single, when it is intensely stained or double with one faint and the other intense zones.

Zone D is the most fast moving region located at about the position occupied by group specific components (Gc). It is faintly stained, comparatively narrow and varies in mobility.

The different groups of cancer present different patterns of these zones.

According to Smith (1968), the normal adult pattern shows 15-25 bands, their positions are characteristic and vary only by a few mm on a 100 mm scale. The albumin band is at 86-100. The main β-globulin band, including transferrin, is around 50, i.e. 48-54. The line of group specific proteins (Gc) is between these two zones. The 0-30 region shows some faint bands representing the haptoglobins.

Group I includes 14 patients with cancers of larynx, pharynx, pyriform fossa and laryngo-pharynx. Most of them show a rather diffuse zone A, variable in size and with little differentiation into more intense bands. Zone B is single and narrow and of more or less similar migration rate. Zone C consists in the cancers of larynx and pharynx of a single intensely stained band, accompanied by a fainter band of the same width in the cancers of pyriform fossa and laryngo-pharynx. Zone D occupies a position between 70-75 and is narrower in two cases. All the cases show uniformity of bands, irrespective of the stage of the disease.

The only two observations made on breast cancer have been placed under Group III. All the four zones are present. Zone A starts from 8-10, zone B is single and narrow, zone C is double with two regions of intensity and zone D occupies a position similar to that of Group I.

Group IV including two cases of cancer of the tongue presents a rather heterogeneous appearance in the width and mobility of almost all the zones. The broad zone A is rather wide, starting nearly at the point of origin and migrating close to zone B. Several narrow intensely stained bands can be identified in this zone. Zone C is double in both the cases.

Twelve cases of cancerous cervix, under Group VII, show a comparatively fast moving zone A, no zone B, wide medium stained zone C and moderately fast D bands.

The pattern for Group IX with one case of oesophageal cancer shows a double stained zone C, a few narrow bands within the undifferentiated zone A and zone B very close to A.
In the last group, Group X, different types of cancers have been included such as of thyroid (1 case), exfoliative dermatitis (1 case) and seminova testis (1 case). Zone C is double in the first case and single in the others. A few intense bands are present in zone A in all three cases and zone B is very close to A in the second case. One remarkable feature is the complete absence of zone D in the first case and a comparatively slow band for the second.

Discussion

As mentioned earlier (Thomas et al, 1976), vertical polyacrylamide gel electrophoresis is effective in presenting a sharp resolution of serum proteins. In many cases of cancer studied earlier, the intensities and position of the different bands could be correlated with the type of cancer concerned, as e.g., the presence of identical patterns in the three cases of Hodgkin’s Disease studied. They differed appreciably from other ones e.g. cases of carcinoma cervix. The main drawback of this method is the occurrence of too many bands which often confuse their identification. However, earlier authors have indicated association of certain characteristic patterns with specific diseases like haematological disorders (Rohlfing et al, 1969), chronic lymphatic leukaemia and lymphosarcoma (Werthamer and Amaral, 1971) and other cancers (Werthamer and Amaral 1976).

In the present series of investigations as well, the cases of a particular type of cancer show similar patterns (Fig. 1). The similarity of the pattern is particularly evident in cancers of larynx, pharynx, pyriform fossa and laryngo-pharynx. In cancer of the cervix where the largest number of cases were observed (12) the zone A is variable ranging from 5 to almost 45 and zone B is absent. This variability of zone A may possibly be due to other factors like the period from the incidence of malignancy. If an equally large number of cases could be studied of other types of can-

CANCER TYPES

Normal Group I Group II Group III Group IV Group V Group VI Group VII Group VIII Group IX Group X

Fig. 1 Protein patterns of human serum following polyacrylamide gel electrophoresis.
cers as well, such variability may be observed. The difficulty of the use of this method in ultimate diagnosis is the presence of such variable factors which may affect the relative intensity and the position of the bands.

Taking in all, however, there is a tendency of the total protein patterns from patients affected with one particular type of cancer to show marked similarity. The precise identification of these bands through other staining procedures has been attempted by the present workers as well as earlier ones. Observations of the transferrin bands after specific staining has not been as effective as the total protein pattern in the identification of the different kinds of cancer (Thomas et al, unpublished). The genetic variants present of the different serum proteins further complicate the issue. Attempts are being made to make an assessment of the different genetic variants of the serum protein present in the malignant cases as compared with the normal population. In the case of haptoglobins for e.g. no relationship has been observed between the Hp1 gene frequency and the incidence of malignancy (Baksi et al, 1975).

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CONCLUSIONS

1. The use of disc polyacrylamide gel electrophoresis shows a clear resolution of the different serum proteins occurring in characteristic patterns as bands of different intensities, size and migration rates in normal individuals. Any particular protein can be identified from its position in this pattern.

2. The same method, on being applied to sera from cancer patients, results in distinctive patterns as well. These patterns again present a general similarity, but vary markedly from that given by the normal individuals. The protein bands can be grouped into four distinct zones, on the basis of their location on the gel. A characteristic feature is the presence of a distinct band in zone C, corresponding to the main $\beta$-globulin, which is wider or double in particular types. The protein in zone A occurs as a wide region of diffuse staining, ranging from almost the point of origin to about 35-40, on a 100 mm scale. It may be differentiated into positive bands in the different forms of cancer. Zone B, located above the main $\beta$-globulin shows diversity both in intensity and position of the bands. Zone D has a rather narrow band occurring at about the position of the Gc group specific proteins. It occupies slightly different locations in this region in the different types of cancer.

3. The patterns are fairly characteristic within a parti-
cular group of cancer, as for example, amongst the cancer of cervix (16 cases) and of larynx (21 cases). These groups, however, must be further categorized before these patterns can be used for final diagnosis.

4. The limitations of the use of this method for diagnosis are:

   (i) certain proteins like haptoglobins (Hp), transferrins (Tf) and group specific proteins (Gc) are present in polymorphic forms which may result in variations in the corresponding band in the total protein pattern.

   (ii) the nature of the cancer, whether it is a primary growth or a secondary one, complicates the observations.

   (iii) sera from cancer patients contain protein fractions that are immunosuppressive. The age of the serum, the duration of the disease and the fact whether it is treated or not are other important factors in assessing the patterns for diagnosis. There is also the possibility that protein variations might have arisen from other diseases or treatments for secondary diseases.

5. Taken in all, however, the method of disc polyacrylamide gel electrophoresis can be employed effectively in diagnosing the incidence of malignancy through the study of total protein patterns. The method must be further developed if the nature of the cancer has to be identified, depending on whether it is of primary or secondary incidence.
SERUM protein patterns have been studied in 102 cases of malignancy from local hospitals by vertical polyacrylamide gel electrophoresis. For normal control, blood was obtained from healthy donors at the local blood bank.

The sera were subjected to disc polyacrylamide gel electrophoresis following a modification of the existing technique. The gel composition was 7.5%, the buffer used was Tris Glycine, pH 8.3 and the run was given at a constant current of 4 mA per tube for 1½ hours. The gels were subsequently stained with 0.1% Amido black in 7% Acetic acid in water for ten minutes followed by destaining in the same solution with the dye. Protein bands appeared as dark blue bands on a faintly stained background.

The patterns presented in the normal individuals were characteristic and differed distinctly from those observed in sera from cancerous individuals.

The 102 cases of cancer were classified under ten groups, based on the location of the malignant growth. The protein bands obtained after disc acrylamide gel electrophoresis formed four zones from the point of origin, designated for convenience as A, B, C and D. The different groups of cancer presented different patterns of these zones. Protein patterns within
particular groups of malignant cases were similar in many groups, and differed appreciably from other groups. This agrees with the observations of earlier workers where the intensities and position of the bands could be correlated with the type of cancer concerned.

This method can be employed effectively to some extent for the diagnosis and prognosis of particular varieties of carcinomas, but it must be further developed if the exact nature of the cancer is to be identified, depending on whether it is of primary or secondary incidence.
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