Changes in Immune Profile in Liver and Gastrointestinal Cancer

BY

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Introduction

I - INTRODUCTION

Cancer is one of the most recalcitrant problems in human heath (Weinberg, 1985). Cancer is the result of multiplication of some cells in the body, without restraint and produces a family of descendants that invade the surrounding tissues (Cairns, 1985). It attacks the basic life process of the cell in almost all instances altering the cells genome and leading to a wild and spreading growth of the cancerous cells (Guyton, 1981). Cancer is a disease that no longer be restricted to the confines of a medical phenomenon alone. Over the years because of its wide spread nature, it has assumed both social and psychological overtones. It comes from insults to the body that in the best of worlds could be avoided (Weinberg, 1985).

According to WHO estimates there are 37 million cases of cancer throughout the world. Irrespective of the country cancer is among the world's three main causes of death (Sward, 1983). 2,50,000 cases of liver cancer makes it eigth on the world wide cancer list although its incidence is essentially high in some areas including Southern China (Crozemarie, 1986).

Primary Carcinoma of the liver is considered to be the second commonest malignancy in South East Asia (Mobin Khan and Islam, 1985). High incidence is seen in tropical climate and in areas where poverty prevails. In India it has been found to be a common tumor in the eastern parts of Uttar Pradesh and neighbouring districts of Bihar (Khandelwal and Khanna, 1986).

Tumors of the alimentary tract are relatively common and are important causes of mortality (Mahanthy et al., 1983). Prabhakar et al., 1981 quoted data of incidence of gastrointestinal malignancies from some parts of India which show variation from place to place.

As early as in 1909, Erlich putforth the hypothesis that host defences may play an important role in preventing neoplastic cells from developing into frank tumors (Gangal, 1979).

When a cell undergoes malignant transformation, it acquires new surface antigens. This makes a tumor antigenically different from normal cells (Ananthanarayan and Paniker, 1983). The immunosurveillance mechanism controls the growth of cells and destroy them and this immune function plays a great role in preventing the onset of malignancy. The immune system act through two mechanisms, that is, antibody mediated immunity and cell mediated immunity.

An important role for immunological process involving both cellular and humoral mechanism in the detection and control of neoplastic disease is being widely studied (Nawalka et al., 1983).

The antibodies involved most commonly are IgG, IgA and IgM. There are alterations in many neoplastic conditions. Immunoglobulin estimation showed high levels of IgG in various hepatic disorders including hepatoma (Malhotra et al., 1986). Hughes (1971) found increased IgA levels in carcinoma of gastrointestinal tract. The values of serum IgM concentration

in cancer patients were not much altered when compared with normal (Agarwal et al., 1984).

Cell mediated immunity as assessed by T cell count was depressed in primary carcinoma of the liver. Depressed cell mediated immunity has also been reported in under nurished patients with cancer of the Oesophagus (Haffejee et al., 1978) and at various sites in the gastrointestinal tract (Burke et al., 198

Leucocyte count is usually raised to about 10,000 per cu mm with 80 per cent polymorphonuclears. Eosinophilia is occassional finding and anemia is mild in most of the cancer patients (Sherlock, 1975).

Lysozyme plays an important role in immunity reactions (Agarwal and Sastry, 1981). Lysozyme is not a typical lysosomal enzyme. It is also found in the granule fraction of mouse polymorphonuclear leucocytes (Dingle, 1977) and rabbit macrophages. The presence of macrophages may be important in the final effector pathyway of antitumor immunity (Coppleson, 1981). Iron also plays a role in the host's resistance to infections, cell mediated immunity has been shown to be impaired in iron deficiency (Bagchi, et al., 1980).

Albumin and vast majority of globulins are made in the liver. The major exception is the immunoglobulins which are made by β-lymphocytes (Plasma cells). Hypoalbuminemia is common in patients with cancer (Raines et al., 1982). The serum globulin level may be normal or increased or even occassionally

very high in the case of liver cancer (Sherlock, 1975).

The likelyhood of cancer developing in respiratory and digestive organs is greater in subjects with a low level of vitamin A (Joyce et al., 1986). The study of vitamin A is also interesting because it has been shown that vitamin A could have an adjuvant role in the immune response.(Zamaria et al., 1985).

No disease in modern history has captured the imagination and fear of mankind as has cancer and AIDS. It continues to generate terror, uncontrollable fears and a hopeless anxiety in its victim. So it is necessary to conquor this disease and prevent early death.

At present cancer is treated in three basic ways, surgery, radiotherapy and chemotherapy. Doses large enough to eradicate cancer with high efficiency can be lethal to the patient and even moderate doses can cause a variety of harmful side effects in the cases of radiation treatment (Collier and Kaplan, 1984). The powerful chemicals that are in use to kill cancer cells also kill or severely injure the normal cells of the body (Davies, 1986). Even when the operation for cancer is curitive, the result may not be due to the removal of every cancer cell but a microscopic residual of tumor which can be eliminated by host's immune mechanism. The immunological defect in the cancer patients is probably a direct result of the malignant state (Manohar, 1984).

There is another way of treatment immunotherapy - the manipulation of immune response, which is still in its infancy

(Bimanesh Sur, 1985). It includes any therapeutic measure intended to strengthen or exploit the immune systems, ability to defend the body against foreign invaders including cancer cells. (Das and Muktar, 1985). Immunotherapy at present is non specific but in the future specific methods should be available (Coppleson, 1981).

Exploitation of tumor immunology for diagnosis, treatment and prevention of cancer recurrences is of paramount importance to the medical community and public at large (ICMR Bulletin, 1979).

The immunity developed in host in response to tumor has opened a new vista for cancer research with respect to diagnosis, prophylaxis and therapy of the disease (Gothoskar, 1979).

Keeping the above points in view, an attempt was made to determine the "Changes in immune profile in liver and gastro intestinal cancers". The study included the determination of serum total protein, albumin, globulin, gamma globulin, immunoglobulin fractions, vitamin A, zinc and white blood cell count, haemoglobin in blood and plasma lysozyme activity.

It is hoped that the findings may in its own way increase the information on immune profile and thus help in the treatment of liver and gastrointestinal cancer by immunotherapy.

Review of Literature

II - REVIEW OF LITERATURE

The review of literature pertaining to the study of 'Changes in Immune Profile in Liver and Gastrointestinal Cancer' is discussed under the following headings:

1. Types of cancer
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1. Types of Cancers

Cancer is not one single disease but a complex of many

diseases. About 200 distinct types of cancer have been recognised. It can be grouped into 4 groups.

(i) Carcinomas are tumours made up principally of epithelial cells of ectodermal or endodermal origin. About 85 per cent of cancers are carcinomas.

(ii) Sarcomas are tumours made up principally of connective tissue cells which are of mesodermal origin, constitute only about 2 per cent of human cancer.

(iii) Lymphomas are cancer in which there is excessive production of lymphocytes by the lymphnode and spleen.

(iv) Leukemias are neoplastic growths of leukocytes (Power, 1983).

Hippocrates of Cos (460-375 BC, Greek Medical literature), the father of Medicine, was the first to divide the tumors into two large groups according to the behaviour.

(i) Innocuous tumors included swelling and lumps of various kinds.

(ii) Dangerous tumors (Malignant neoplasms) are those which killed the patients.

Primary liver cancer includes,

(i) Hepatocelluar carcinoma (relatively common)

(ii) Cholangio carcinoma (rare)

(iii) Angio sarcoma (very rare) (WHO, 1983)

Gastric cancer can be divided into two types.

(i) Diffuse

(ii) Intestinal on the basis of their histology, cytology, mode of growth and secretion of mucin. WHO classification of stomach cancer.

(a) Epithelial tumors

(b) Non-epithelial tumors

(c) Carcinoid tumors

(d) Tumors of lymphoid and haematopoietic tissue

(e) Miscellaneous, matastatic and unclassified (Hill, 1984).

2. EPIDEMIOLOGY

Peculiarities in the distribution of cancer may be due to host factors such as sex, age, race family or predisposing disease or to environmental factors such as diet, occupation, geography, drugs or personal habbits (McConell, 1978).

Cancer is one of the ten leading causes in our country and is advancing in rank year by year (Agarwal et al., 1984). Esophageal carcinoma is frequent in India with an incidence of 14.4 and 11 per 1,00,000 males and females respectively (Dinesh et al., 1981). Variations in death rates from country to country are greater for oesophageal cancer than for any other Gastrointestinal cancer or infact any other tumor. In north Iran an extremely high incidence of oesophageal cancer is observed (Kmet and Mahoubi, 1972).

Mortality from stomach cancer increases sharply with age and roughly twice as common in men as in women of the same age group. In the case of stomach cancer morbidity and mortality patterns likely to be similar because of the relatively short survival time (Water house, 1984).

Death rates from colon cancer are highest in Industrial societies with the notable exception of Japan. The rate of Colon and Stomach cancer are inversely related to each other. Gastric Cancer inspite of declining frequency in the West, is still the second most common cancer in males in Britain and still heads the list in most of Eastern Europe, The Soviet Union, Japan and Chile (McConell, 1978).

Primary liver Cancers account for twenty to thirty percent of malignant tumors found at autospy is Africa and Asia. But they account for only 1-2 Per cent in North and South America and Europe. Liver cancer occurs two or four times more frequently in men than in women. The Peak incidence occurs in the fifth and sixth decade of life in the United states (Lim and Bongard, 1984).

3. CHARACTERISTIC FEATURES OF CANCER CELLS

The cancerous cell generally retains the structural and functional characteristics of the normal cell type from which it is derived. However, they differ from their normal counter parts in several respects.

(I) IMMORTALISATION

Normal cells donot survive indefinitely but cancerous cells can (Power, 1983).

(II) LOSS OF CONTACT INHIBITION

When normal cells make contact with neighbouring cells, junctions are formed and there is slow down of movement. The rate of cell division decreases and also gradual inhibition of cell growth is noticed. Cancer cells behave quite differently (De Robertis and De Robertis, 1981).

(III) REDUCED CELL ADHESION

When normal cells become cancerous there is a change in the stickiness of their cell membranes.

(IV) INVASIVENESS

One of the most important characteristics of transformed cells is their invasiveness. i.e., the ability to invade other tissues (Metastasis).

(V) LOSS OF ANCHORAGE DEPENDENCE

Most normal cells must be attached to a rigid substratum (i.e., they must be anchored) in order to grow. Transformed cells can grow even when they are not attached to the substratum.

(VI) SELECTIVE AGGLUTINATION BY LECTINS

In normal cells the receptors or agglutinin binding sites

for lectins lie in a diffuse manner on the cell surface and are immobile. Lectins make few intercellular bridges and therefore agglutination is not possible. In transformed cells the receptors are more mobile. Within the membrane and so lectins are able to form intercellular bridges to result in agglutination.

(VII) DISORGANISATION OF THE CYTOSKELETON

Normal cells have a cytoskeleton (very much like muscle fibres) which consists of microtubules and microfilaments. These fibres have a regular arrangement and bring about co-ordinated cell movement. In transformed cells, the cytoskeleton undergoes depolarisation. The disorganisation of the cytoskeleton also affects the cell surface.

(VIII) INCREASED SUGAR TRANSPORT

Tumor cells consume much glucose than normal cells because they have to grow and multiply.

(IX) DEFECTIVE ELECTRICAL COMMUNICATION

Electric connections normally occur between individual cells. In some Cancer cells however, it has been reported that such connections are defective (Power, 1983).

4. ETIOLOGY

A. INTRINSIC FACTORS

a. Heredity

The genetic back ground or determination in human cancer is

usually difficult to seperate from the various environmental influences. In experimental animals, however there is a strong evidence that genetic factors play an important role in influenzing the incidence of both spontaneous and experimentally induced tumors.

Multiple polyps of the large intestine, usually cancer of the colon also develops in early adult life (Rosenberg, 1985).

b. Age

Although tumor appear in any age group they tend to occur in old individuals.

c. Sex

Men are affected by Gastrointestinal Cancer more frequently than women (Willis, 1977).

d. Immunologic factors

There are instances also in which there is an apparent association between an increasedcancer incident and a decreased or altered effectiveness of the host's immune mechanism.

Moreover immunodeficiency states either genetically inherited or the result of suppression of the CMI mechanism are associated with an increased cancer incidence and with cancer that grow more rapidly than in general population.

The presence of circulating blocking factors (tumor antigens, or Antigen - Antibody complexes) may prevent sensitised lymphocytes

from attacking tumor cells effectively, whereas a weak immune response, such as that present during the initial stages of tumor development may have a stimulatory rather than an inhibitory effect on tumor growth.

Enhancement of the host immune mechanism by nonspecific stimulants such as BCG vaccine and Cornyebacteruim parvum or the development of unblocking antibodies by the host, may assist in restraining tumor growth and at times in tumor rejection at both primary and metastatic sites. (Diamandopulos and Meissner, 1985).

e. Dietary factors

In studying epidemology of malignant tumors of the mouth, larynx, oesophagus, stomach and liver, diet of the population should be considered including frequency of meals, temperature of food (hot or cold), use of alcohol, dental health. (Blokhin and Chaklin, 1985).

The cancer - diet nexus

Cancer	Dietary factors
Oesophagus	Low intake of meat, fish, fruits and vegetables, alcohol, tobacco.
Stomach	Pickled vegetables, salted substances, fried foods, smoked fish, soyabean, over nutrition.
Colon and rectal	Low fibre.
Liver	Alcohol (Malini Seshadri, 1986).

f. Parasitic factors

Metazoan parasites are associated with cancer in man (Park and Park,1977 and Powar and Danginawala, 1982).

Schistosoma haematobium	-	Carcinoma of the liver.
Schistosoma japonicum	-	Carcinoma of the liver and rectum.
Schistosoma mansoni	-	Carcinoma of rectum.

g. Genetic factors

Cancer genes have been discovered in the chromosomes of tumor cells. These genes, often called oncogenes represent the driving force behind the uncontrolled growth of many cancer cells. It is these genes that become activated in the conversion of the normal founder cell into a cancer cell. Once activated the genes function continously to direct cell toward the abnormal behaviour that is called cancerous state (Weinberg, 1983).

h. Mechanical factors

Chronic gastritis, Polyposis, atrophic gastritis, chronic gastric ulcer, are believed to be aetological factors in gastric cancer (Manohar, 1984). Cirrhosis, hepatitis are the predisposing factors for liver cancer (Khandelwal and Khanna, 1986).

B. EXTRINSIC FACTORS

a. Physical factors

The physical agents which are carcinogenic are ultra violet rays.

b. Chemical factors

Stomach cancer is increased in a number of occupations example coal and petroleum Refining Industries (Rothman, 1980).

Heavy exposure to any one of several different sort of air contaminants increases the risk of cancer, example include tobacco smoke, benzopyrene (BaP), vinyl chloride, radon gas and dust composed primarily of asbestos fibers, radioactive materials or chromates. A large proportion of all human cancers may result from exposure to two or more different agents. One of the agent taken alone may be either a non carcinogen or a very weak carcinogen. Aflatoxin is found to be a liver and oesophageal carcinogen and nitrates, nitrites and nitrosoamines, as stomach carcinogens (Hammond and Garfinkel, 1980).

c. Biological factors

(i) Parasites

(ii) The strongest association is reported by Hepatitis B virus and liver cell carcinoma (Fred Rapp, 1980). Viruses have several ways to change a normal cell into a malignant state (Righy and Wilkie, 1985).

d. Cocarcinogens

Antimetabolites may act as cacinogens perhaps through their immuno suppressive effects (Roit, 1980).

5. BIOCHEMICAL CHANGES

Although many bodily functions show rhythmic changes during a 24 hours period, it seems the tumors donot have rhythmic metabolic processes (Davies, 1986).

Tumor cells donot have specific enzymes, proteins or metabolic changes that are common to all tumors nor are such changes consistently differ from normal cells. There is a great diversity of biochemical characteristics of both normal and neoplastic tissue and as yet no differences are constant or specific enough to be of diagnostic value. Tumor enzyme patterns however do tend to resemble each other more than these of normal tissue. Although in most instances neoplastic cells expand their energy on proliferation, rather than on production, after they make various substances in varied amounts either intra or extracellularly (Kissane, 1985).

From the chemical point of view, cancer cells compared with normal cells have

(1) Lower pH
(2) A greater free radical character
(3) Tumor produced hormone peptides
(4) Tumor associated antigen
(5) A low ca^{++} concentration and high K^{+} concentration.
(6) Elevated amounts of methylated nucleosides
(7) A high concentration of plasma mucopolypeptides
(8) Higher biowater content.
(9) A greater need of exogenous zinc (Bimanesh sur, 1985)

There may be a disturbed salt metabolism in some patients who have carcinoma of the stomach.

(a) Protein metabolism

Theory suggests that cancer may be the result of a loss or an alteration of proteins that although essential for control of growth are not essential for survival. Many changes is reported in cancer patients such as a deficiency in specific proteins or the presence of abnormal proteins.(Meissner, 1985).

Muscle wasting in the patient with cancer is clear evidence of an altered protein metabolism (Schersten et al., 1982). The energy costs of the body seem to account only for about 20% of the negative energy balance of the tumor host (Raines et al., 1982).

Since increased coagulability of the blood may complicate cancer particularly those of Pancreas and stomach. Blanchard et al. in 1983 detected carboxy Prothrombin, an abnormal Prothrombin, in the serum of 69 of 76 patients (91 percent) with hepato cellular carcinoma. Normal subjects have no detectable abnormal prothrombin in their blood. In contrast, levels of normal prothrombin were low in patients with chronic active hepatitis or metastasis carcinoma involving the liver (Clark et al., 1983).

The histamine concentration in patients with malignant tumors were low, as compared with the levels in other groups of non malignant diseases. In patients with malignant disease the mean histamine values did not vary according to the organ involved

or the histologic features. Levels in patients with malignant disease who were treated with chemotherapeutic agents or irradiation were significantly lower than those in untreated patients. In patients with stomach cancer, the histamine levels tended to rise after resections for cure (Motoki et al., 1984).

In severe hepatocellular disease the plasma level of albumin, and many other plasma proteins fall. The rise of plasma aminoacids that follows ingestion of a meal containing protein stimulates the liver to synthesize albumin. Inflammation stimulates the synthesis of a number of plasma proteins and in so doing appears to divert the protein synthetic apparatus of the hepatocyte away from the synthesis of albumin. For this reason plasma albumin levels fall in all chronic diseases not just in those disorders directly affecting hepatocytes.

Hypoalbuminemia is common in patients with cancer and may be the result of decreased synthesis, increased metabolism, increased plasma volume, or the passage of protein rich fluid into the gut, protein losing enteropathy. Gastric carcinoma is one of the diseases characterised by increased protein loss into the gut (West, 1985). Raines et al.(1982) emphasized the importance of an understanding of the effects of nutrition on albumin metbolism because of its critical importance in relation to drug transport and actions.

Hypoalbuminemia in metastatic tumors of liver was reported by various authors. It was found that in the liver of animals

with Zajdela hepatoma, the albumin synthesizing apparatus is substantially reduced. i.e., the proportion of polyribosomes on which its polypeptide chain form sharply diminishes constituting only 5-70 per cent of their content in normal liver while the total content of polyribosomes does not change (Shapot and Berdinskikh, 1975).

(ii) Globulins : Immunoglobulins

Pretherapy IgG level went down while IgA level rise and the serum level of IgM was not seem to be altered in cancer patients as compared to controls. Post surgery serum immunoglobulin levels had altered but not very much, though there was a marked reduction in serum immunoglobulin levels after radiotherapy and chemotherapy (Agarwal et al., 1984).

Smith (1972) found lower values of IgG found in cancer patients may be related to antibody production capacity in malignancy to tetanous toxoid, Lee et al. (1970) found deficiency in IgG antibody to Flagellin in patients with active cancer.

Hughes (1971) found increased IgA levels in carcinoma of oral cavity, Skin, Gastro intestinal tract and lung. Brown et al. (1975) and Cochran (1978) have reported increased IgA levels in different malignancies while Hughes (1971) suggested that increased IgA could be due to infection of the secreting mucosal epithelium as seen in carcinoma of lung and gut.

The values of Serum IgM concentration in cancer patients were not much altered when compared with normal. It was reported

by Robert et al, (1975) and Wang et al, (1977) the lack of alteration in serum IgM levels may be due to the fact thath the plasma cell containing IgM are less in mucosa of oral cavity, gastrointestinal tract and breast epithelium (Hughes 1971). Therefore there is no appreciable difference in local production of IgM due to cancer tissue and subsequent absorption in the serum.

The depression of Immunoglobulin levels after radiotherapy and chemotherapy can be explained on the basis that both act on lymphoreticular haemopoetic system which is essential for maintaining immunity.

The cytotoxic drug acts by demaging the capacity of the body to produce an immune response thus lowering the serum immunoglobins (Evelyn and Walter, 1969). The radiotherapy has more drastic effect on the serum immunoglobin levels than the chemotherapy. This can be explained on the basis of hypothesis by David Grey (1970) i.e., when an antimalignant drug is administered it suppresses the transformation of lymphocytes, which are responding to the antigens. This imparts a specificity of action not obtained with radiation which destroys antigen reactive lymphocytes and bone marrow cells indiscriminately.

Tumor antigenic load might be playing a great role in immunoglobins levels. The removal of tumor tissue by radio therapy probably leads to drop in levels of Antibody gradually. The changes after therapy may be utilised for monitoring the therapy on patients. In liver carcinoma, serum IgG and IgM levels were within the normal range. Hughes (1971) also reported no significant

difference in IgG levels in patients of Gastrointestinal ulcers.

IgA levels were found to be significantly high in primary hepatocellular carcinoma and cirrhosis patients as compared to healthy controls. In metastatic carcinoma of the liver, there is no significant alteration in IgA levels (Agarwal et al., 1984).

The increase in IgA levels in primary carcinoma but not in secondary carcinoma could be because 75% of the primary carcinoma patients in their study had already post necrotic cirrhosis. IgA has a neutralising effect against viruses and antigenic stimulation of hepatitis - B virus in the liver results in an increased synthesis of IgA with consequent increase in its concentration in the serum (Khandelwal and Khanna, 1986).

Total globulin levels were raised in patients with diverse hepatic disorders including carcinoma (Malhotra, et al., 1986).

(iii) Enzyme levels

A variety of enzyme markers have been proposed for the early detection of cancer for following the progression of the disease and identifying individuals at high risk.

In cancer cells there is an ordered pattern of quantitative and qualitative imbalence in enzymology linked with malignant transformation and progression (Clark and Cumley, 1978).

Lysozyme

In 1922, Alexander Fleming, a bacteriologist in London discovered the antibacterial substance lysozyme. He also discovered

a small round bacterium that was particularly susceptible to lysozyme which he named Micrococcus lysodeikticus (Stryer, 1981). The lysosomes contain bacteriocidal agents that can kill phagocytized bacteria before they can cause cellular damage. They include lysozyme that dissolves the bacterial cell membrane (Guyton, 1981). Almost all human tissues and fluids contain lysozyme. Tears are the rich source of lysozyme. Lysozyme is found in macrophages and is a good marker for macrophages, animal and human studies have suggested that macrophages play an important role in host defence against neoplasms (Nomori et al., 1986).

High concentration of Lysozyme in urine or serum found in almost all cases of acute monocytic, acute myelomonocytic Leukemia (Miale, 1982 and Hoffbrand and Lewis, 1981).

(b) Vitamins : Vitamin A

Vitamin A and β carotene have been shown to have a protective effect on cancers of larynx, oesophagus, bladder, lung, stomach etc. (NIN, 1985, Stehr et al., 1985 and Hennekens, 1986).

Also a growing accumulation of epidemological evidence indicate that there is an inverse relationship between the risk of cancer and consumption of foods that contain vitamin A or its precursors (eg. liver, green and yellow leaf vegetables) (Wald et al., 1980, Hiat et al., 1982, Willet et al., 1984 and Joyce et al., 1986). Synthetic retinoids are used to control tumor growth (Krishnamoorthy, 1986).

(c) Minerals : Zinc

Zinc is known to be protective against oral and other Gastrointestinal cancers (NIN, 1985). In non-cancer populations, zinc has been shown to play a role in maintaining normal appetite, taste, and immunocompetance (Lindsey and Piper, 1986).

The blood and tissue levels of zinc in cancer patients are lower (Giri et al., 1984).

6. IMMUNOLOGICAL ASPECTS IN CANCER

The immuno surveillance aspect of the immunological system has wide applications in our body. Some of the newly formed cells are prone to have an occassional genetic abnormality. If the cells are permitted to grow they may result in tumor formation. The immunosurveillance mechanism controls the growth of cells and destroys them and this immune function plays a great role in preventing the onset of malignancy. In patient with immunological disorders, there are very high rates of neoplasia (Kersey, 1974, Penn, 1974 and Grosser, 1976).

Evidence that cancer patients may have impaired immunologic responsiveness that could influence the course of their diseases, gives importance to studies of immunologic competance in such patients (Nawalka et al., 1983).

The specificity of the immunity was to the individual tumor concerned and not to other sarcomas even those raised by the same carcinogen in the same mouse (Lachmann, 1984).

(a) Antibody mediated immunity

The antibodies involved most commonly are IgG, IgA, IgM. The levels of immunoglobulins are constant in health but there are alterations in these levels in many neoplastic conditions (Cochran, 1978).

(b) Cell mediated immunity (CMI)

Cell mediated immunity as assessed by T cell count, was depressed in Primary Carcinoma of the liver. Immune response in Uganda patients with Primary cancer of liver was reported to be negative. Gupta et al. (1980) who studied delayed cutaneous hypersensitivity in 8 primary liver cancer patients also reported depressed immune response (Khandelwal and Khanna, 1986).

(c) Haematological changes

Higher mean total leucocyte count were detected in cancer patients (Leucocytosis is seen) (Kapoor et al., 1983). Lymphocyte count is inversely correlated with tumor stage and is an aid to prognostication in the treatment of patients with neoplasms (Desai, 1983). Eosinophils are part of the immunological reactions requiring the presence of antigens, macrophages and thymus dependant lymphocytes. One of functions of eosinophils is perhaps a processing of Antigen-Antibody complexes. There was a progressive rise in the eosinophil count noticed in study of Srivatsava et al. (1983).

(d) Tumor Antigens

Tumor antigens may be divided into 3 classes.

(i) Those unique for one single tumor
(ii) Those shared between a number of tumors
(iii) Antigens shared between a given tumor and normal cells than the cell of tumor origin in the host. A given tumor may express all three classes of antigens (Nathrath, 1978). These antigens not preciously recognised or not produced become detectable on the cell surface, as the neopolastic cell arises from the normal cell, known as Tumor Specific Antigen (TSA) or Tumor Specific Transplantation Antigen (TSTA). The constant expression of an identifiable antigen is a useful diagnostic aid and has been used to suggest that a tumor of unknown cause is of viral origin. Antibodies to TSA antigen are found in tumor bearing animals (Barrett, 1983).

(e) Tumor markers

Many immunologic substances and other proteins detectable in the circulation are associated with human cancer. They are used for detection (scanning in asymptomatic persons) diagnosis (differentiating malignant from benign conditions), monitoring, classification, staging (defining the extent of disease), localisation and therapy (cytotoxic agents directed to marker containing cells).

CLINICALLY USEFUL TUMOR MARKERS

(i) Carcino Embryonic Antigen (CEA)

One of the best tumor markers will increase in stomach cancer. CEA is present in extracts of tumors from patients with adenocarcinoma of the large bowel as well as endodermal tissues during the first two trimesters of fetal development.

Since CEA clearence occurs chiefly in the liver, the highest CEA values have been noted in patients with liver metastases from colorectal cancer.

CEA elevations have also been reported in a variety of benign diseases and disorders. However, non-malignant conditions usually produce transient and modest elevations in CEA (Mcintire, 1984 and Desai et al., 1984).

(ii) Alfa fetoprotein

The immune reactions are often Quite weak and since the immune mechanisms may have adequate access only to the outside of the growing tumor. Weak immune responses may stimulate the growth of tumors rather than suppress it. Alfa fetaprotein is a well known tumour product that can be demonstrated to have immunosuppressive activity (Lachmann, 1984).

It is synthesized during normal pregnancy in the foetal liver and Yolk Sac, reaching a very high concentration. Its name derives from this fetal origin. Alfa refers to its electrophoretic migration characteristics (Ann Light, 1985).

High levels occur in primary hepato cellular carcinoma (Klee and Go, 1982 and Gorman et al., 1985). Cases of Secondary Carcinoma of liver gave negative results for serum AFP (Agarwal et al., 1984).

(iii) Australia antigen or Hepatitis B surface Antigen

It is known to present in cirrhosis of the liver, hepatoma (Patel et al., 1981 and Sharma and prasad, 1985).

Hormones and immunity

Any hormone that is produced by a neoplasm which is derived from tissue not normally engaged in the production of the hormone in Question, is described as atopic hormone (Liddle, et al., 1969). One such example is human chorionic gonadotropin (HCG) from hepatic tumors causing prococious puberty (McArthur et al., 1973). The hormone production may be of importance for tumor survival. The recently reported effects of HCG (Adock, 1973) and of the opiods (Weber and Pert, 1984) on the immune system may well contribute to the survival of tumor cells that might be otherwise destroyed as non-self (Howlett and Rees, 1985).

(f) Role of immunity in Gastric Carcinoma

The role of immuno therapy in gastric carcinoma is still not well established. The large the tumor mass the greater the immunodepression. Surgery removes the cancer cells and lowers tumor burden which can alter the immune balance to favour the patient (Manokar, 1984).

7. EMERGING AREAS OF RESEARCH

One of the goals of cancer reserach is to ascertain the mechanism of cancer (Dulbecco, 1986). Since cancer cells that become resistant to one drug frequently become resistant to several other unrealated ones (Science, 1986). A new approach to treat metastatic cancer is adoptive immunotherapy, a treatment in which immune cells with antitumor reactivity are transfered to the tumor bearing host (Rosenberg et al., 1986) is adopted. Also studies are going on regarding antibodies, since anti-idiotypic antibody bear the internal image of a human tumor antigen (Hirlyn et al., 1986).

Experimental Procedure

III-EXPERIMENTAL PROCEDURE

The experimental procedure adopted to investigate the "Changes in Immune profile in Liver and Gastrointestinal Cancer" is presented below.

1. Selection of patients
2. Distribution of the controls and patients
3. Collection of blood samples
   (a) Colection of blood from the patients by venipuncture
   (b) Preparation of oxalate bottles
   (c) Separation of serum
4. Estimation of biochemical para meters
   (i) Serum total protein, albumin, globulin and gamma globulin
   (ii) Serum Immunoglobulins IgG, IgA and IgM
   (iii) Serum vitamin A
   (iv) Serum zinc
   (v) Total and differential count of white blood cells
   (vi) Haemoglobin
   (vii) Plasma Lysozyme activity

1. Selection of patients

Thirty liver and gastrointestinal cancer patients who were undergoing treatment during January-February, 1987 in Coimbatore Medical College Hospital, Kuppuswamy Naidu Memorial Hospital, Coimbatore and Employees State Insurance (ESI) Hospital, Singanallur were selected for the study. They were in the

age group of 30-70 years and of both sexes. Thirteen normal, healthy individuals of the same age group and those who were suffering from liver and gastrointestinal non neoplastic disorders of same age group served as controls and diseased controls respectively for comparison.

2.Distribution of the controls and patients

The controls, disease controls and cancer patients selected for the study were grouped as shown in Table I.

TABLE - I

DISTRIBUTION OF THE CONTROLS, DISEASE CONTROLS AND CANCER PATIENTS BASED ON THE DISORDER AND SEX

Group No.	Disorder	Sex		Total
		Males	Females	
I	Healthy Normal controls	7	6	13
II	Non malignant liver disorders			
	(a) Jaundice	6	-	6
	(b) Cirrhosis	3	2	5
III	Non malignant GI tract disorders			
	(a) Peptic ulcer	2	2	4
	(b) Oesophagitis	1	2	3
IV	GI tract cancer			
	(a) Oesophagcal cancer	5	6	11
	(b) Stomach cancer	2	2	4
	(c) Intestinal cancer			
	(i) Small intestine	2	-	2
	(ii) Rectum and anus	3	1	4
V	Liver cancer			
	(a) Primary	4	1	5
	(b) Secondary	2	2	4

3. Collection of blood samples

(a) Collection of blood from the patients by venipuncture

The blood was collected as follows (Oser, 1976).

Tied a tourniquet (or soft rubber tubing or strip of bandage) tightly around the arm of the patient, a couple of inches above the elbow. Had the subject clench his fist firmly, washed the skin surface about the prominent vein on the inner surface of the elbow (usually median basillic) with seventy per cent alcohol, allowed to dry, held the vein immobile by pressing on it with the thumb below the elbow and into the vein, inserted a sharp, sterile hypodermic needle(No:22) an inch and a half long which was attached to a dry, sterile syringe of suitable capacity. The needle should penetrate the vein from the side end, at an angle of 50° with the surface of the arm, the level or opening of the needle being kept upwards or to the side. As soon as blood was seen to enter the syringe, retracted the piston slowly until the desired amount of blood had entered the syringe. Before removing the needle from the vein, loosened the tourniquet, had the patient unclench his fist and on the skin, at the point of entrance of the needle, held in place a small pad of folded gauze moistened with seventy per cent alcohol. Withdrew the needle, detached it from the syringe (not too vigorously which might cause hemolysis) and then transferred the blood to a centrifuge tube.

5.0ml blood was distributed in two tubes as follows.

1) 3.5 ml of blood was used to seperate serum in a clean non-oxalated tube,
2) Remaining blood was oxalated to seperate plasma.
Whole blood, needed for the estimation of haemoglobin and the total and differential counts of white blood cells was taken from this protion.

b) Preparation of oxalate bottles(Heller and Paul, 1975)

1-2g of Ammonium oxalate and 0.8g of potassium oxalate were dissolved in 100ml of water. 0.1 ml of this solution was used for collecting 1.0ml blood. The oxalate solution was dried before blood was added to the tube and mixed well.

c) Seperation of Serum (Tietz, 1976)

After the blood was drawn from patient by using a syringe, it was immediately transferred to a clean dry tube after the needle has been removed. The blood was then allowed to clot for atleast 10-15 minutes at room temperature. The clot may adhere to the wall of the tube so that 'ringing' (making a gentle sweep around the inside walls of the tube with a wooden applicator stick) should be performed before centrifugation. Excessive ringing is unnecessary and can produce hemolysis. By allowing the clot to retract for a long period of time hemolysis is minimised and the yield of serum is greater. After the blood had clotted, the tube

was centrifuged and the supernatant serum was removed.

4. Estimation of Biochemical parameters

(i) Serum total protein, albumin, globulin, gamma globulin were estimated by biuret method (varley, 1975).

The levels of various serum proteins are normally altered in cancer patients, especially in liver cancer. This is due to the fact that most of the proteins are synthesized in the liver (West. 1985).

The details of the method are given in appendix - I.

(ii) Serum Immunoglobulins IgG, IgA and IgM were estimated by Tripartigen - Immuno diffusion method (Behringwerke, 1986).

The idea that tumor cells bear Neoantigens, which can in principle elicit an immune response, is now widely accepted (Skornick et al., 1986). This results in altered immunoglobulin levels in the sera of patients.

The details of the method are given in appendix - II.

(iii) Serum Vitamin A was estimated by trifluoroacetic acid method of Gyorgy and Pearson (1967).

In experimental animals, vitamin A dificiency has been shown to lead to premalignant changes in the respiratory and gastrointestinal cancers. Vitamin A also has a adjuvant role in immune response (Wald et al., 1980).

The details of the method are given in appendix - III

(iv) Serum zinc was estimated by the method of Piper (1966).

Low zinc levels have been linked to risk of cancer (NIN, 1985).

The details of the method are given in appendix - IV.

(v) Total and differential count of white blood cells were done by Truck's fluid method and Leishman stain method respectively (Samuel, 1986).

Higher mean total leucocyte counts were detected in patients with tumors compared to normals. Recent reports indicate that lymphocyte count is inversely correlated with tumor stage (Desai, 1983).

The details of the method are given in the appendix - V

(vi) Haemoglobin was estimated by the method of Cart Wright(1958).

Low mean haemoglobin concentration is observed in malignant tumorbearing patients (Kapoor et al., 1983).

The details of the method are given in the appendix - VI

(vii)The Lysozyme activity was estimated by method of Harrison (1968).

Lysozyme plays an important role in immune response (Agarwal and Sastry, 1981).

The details of the method were given in appendix - VII

Results and Discussion

RESULTS AND DISCUSSION

The present study aimed at assessing the "Immune profile in liver and gastrointestinal cancer". One thousand cancer patients were screened during the study period. Of those cancer patients sixteen and three hundred were found to be suffering from liver cancer and GItract cancer respectively. Nine liver cancer patients and twenty one gastrointestinal cancer patients were taken for the study.

The results of this study are discussed under the following headings:

A. Prevalence of the different types of cancer

B. Age and sex composition of the patients

C. Changes in the biochemical parameters in normal controls, diseased controls and in cancer patients

(i) Serum albumin, globulin and albumin globulin ratio

(ii) Serum total protein and gamma globulin levels

(iii) Serum immunoglobulin levels

(a) IgG (b) IgA (c) IgM

(iv) Serum vitamin A level

(v) Serum zinc level

(vi) Total and differential count of Leucocytes

(a) Total count and polymorphonuclear cells

(b) Eosinophils and lymphocytes

(vii) Heamoglobin levels and

(viii) Plasma lysozyme activity

D. Comparison of primary and secondary cancer of liver

E. Comparison of different types of the gastrointestinal cancer

(a) Stomach and oesophageal cancer

(b) Stomach and intestinal cancer

(c) Oesophageal and intestinal cancer

A. PREVALENCE OF DIFFERENT TYPES OF CANCER

One thousand cancer patients were screened during the months January-February, 1987, in Kuppuswamy Naidu Memorial Hospital, Pappanaickenpalayam, Employees State Insurance Hospital, Singanallur and Coimbatore Medical College Hospital, Coimbatore. Most of these patients were admitted in Kuppuswamy Naidu Memorial Hospital for the radiotherapy which was not available at the other two hospitals.

Among the cancer patients screened in the above said hospitals, oral cancer was found to be highly prevalent, following oesophageal cancer. Cancer of liver and alimentary tract cancer other than oesophageal and oral cancer were relatively rare.

The prevalence of the different types of cancer was found to be as shown in Table II.

TABLE - II

PREVALENCE OF THE DIFFERENT TYPES OF CANCER

Type of cancer	Number of Patients	Percentage of prevalence
Cervival and Uterine cancer	425	42.5
Gastrointestinal cancer	300	30.0
Breast cancer	186	18.6
Cancer of larynx	25	2.5
Leukemia	20	2.0
Liver cancer	16	1.6
Cancer of thyroid	10	1.0
Lung cancer	10	1.0
Brain cancer	8	0.8

As seen from the table - II, in the present study among the patients screened, cervical cancer was found to be highly prevalent (42.5%). This was followed by GI tract cancer, (30%) Breast (18.6%), Cancer of larynx (2.5%), Leukemia (2%), Liver cancer (1.6%), Cancer of thyroid (1%), Lung cancer (1%), and Brain cancer (0.8%).

B. AGE AND SEX COMPOSITION OF THE CANCER PATIENTS

Age and sex of the cancer patients screened are presented in Table - III.

TABLE - III

AGE AND SEX COMPOSITION OF THE CANCER PATIENTS

Age (In years)	Sex	
	Male	Female
Below 20	3	1
20 - 30	-	4
30 - 40	28	85
40 - 50	259	400
50 - 60	22	180
60 - 70	10	8
Total	322	678

From the table - III it was observed that old people were the common victims of cancer, most of the patients belong to the age group of 40-60 years. Weinberg (1985) Quotes 'Cancer is in large part as affliction of old'.

Of the patients screened, cancer was more prevalent among females due to the high incidence of cervical, uterine and breast cancer. However, liver and gastrointestinal cancer were more common in males. Willis (1977) also has reported a similar finding.

C. CHANGES IN THE BIOCHEMICAL PARAMETERS IN NORMAL CONTROLS DISEASED CONTROLS AND IN CANCER PATIENTS

(i) Serum albumin, globulin levels and albumin globulin ratio

Table IV shows the mean serum albumin and globulin levels in controls, disease controls and in different types of cancer.

TABLE - IV

MEAN SERUM ALBUMIN AND GLOBULIN LEVELS IN

NORMAL CONTROLS, DISEASE CONTROLS AND IN CANCER PATIENTS

Group	Types	No	Albumin (g/100ml) Mean ± S.D	Globulin (g/100ml) Mean ± S.D	Groups compared	't'-values
A	Normal controls	13	4.30±0.31 (A_1)	2.18±033 (A_2)	A_1 Vs B_1	10.870**
					A_1 Vs C_1	1.706NS
B	Nonmalignant Liver disorders	11	2.94±0.27 (B_1)	3.98±0.26 (B_2)	A_1 Vs D_1	14.126**
					A_1 Vs E_1	11.250**
C	Nonmalignant GI disorders	7	4.06 ±0.23 (C_1)	2.98±0.66 (C_2)	B_1 Vs D_1	4.385**
					C_1 Vs E_1	7.109**
D	Liver cancer	9	2.36±0.26 (D_1)	3.59±0.43 (D_2)	D_1 Vs E_1	4.486**
E	GI cancer	21	2.99±0.36 (E_1)	4.26±0.35 (E_2)	A_2 Vs B_2	14.025**
					A_2 Vs C_2	3.426**
					A_2 Vs D_2	8.286**
					A_2 Vs E_2	17.449**
					B_2 Vs D_2	2.373*
					C_2 Vs E_2	6.307**
					D_2 Vs E_2	4.323**

**- ($P < 0.01$)

* - ($P < 0.05$)

NS- NOT SIGNIFICANT

The mean serum albumin levels of normal controls, non neoplastic liver disease controls, non neoplastic GI tract disease controls, liver cancer patients and GI tract cancer patients were found to be 4.30 ± 0.31, 2.94± 0.27, 4.06 ± 0.23, 236 ± 0.26, 2.99 ± 0.36g/100ml respectively and the individual values are given in appendix - VIII and illustrated in figure-1.

According to Varley (1980) the normal range of serum albumin level for adults is 3.6-5.0g/100ml. Based on this criterian, the mean serum albumin levels of the non malignant liver disease controls, and both liver and GI tract cancer patients were found to be low. It was found to be significantly ($P < 0.01$) lower than that of the normal controls selected for the study.

Comparison of the albumin levels of the patients with liver and GI tract cancer showed that it is significantly ($P < 0.05$) lower in liver cancer patients than in GI tract cancer patients. It was also found to be significantly ($P < 0.01$) lower in liver cancer patients than in patients with non malignant disorders of liver. The low albumin values found in GI cancer patients were found to be significant ($P < 0.01$) when compared with that of patients with non malignant GI tract disorders.

Albumin is synthesized in the liver. In severe hepatocellular disease, theserum level of albumin falls. Inflammation of the liver stimulates the synthesis of a number of plasma proteins and in doing so appear to divert the protein synthetic apparatus of the hepatocyte away from the synthesis of albumin. For this

reason plasma albumin levels fall in all chronic diseases (West, 1985).

The results of low albumin levels in patients with liver cancer and in non malignant disorders of liver are consistent with the observations of Waldman et al. (1974) and Shapot and Berdinskikh (1975).

The mean serum globulin levels were found to be 2.18 ± 0.33, 3.98 ± 0.26, 2.98 ± 0.66, 3.59 ± 0.43, 4.26 ± 0.35g/100ml in normal controls, non malignant liver and GI tract disease controls, liver and GI tract cancer respectively and the individual values are given in appendix-VIII and illustrated in figure-1. As reported by Nancy roper (1985), the normal range of serum globulin level for adults is 1.80 - 3.20g/100ml. Based on this, a state of hyperglobulinemia was noticed in non malignant liver disease controls as well as in cancer patients, whereas it was found to be within the normal range in normal controls and non malignant GI tract disorders.

The difference in globulin content of the different patients was found to be statistically significant when compared with that of the normal controls and also that of different groups of patients. A significant rise ($P < 0.01$) in serum globulin levels was observed in patients with non malignant liver disease on comparing their levels with those of the liver cancer patients. The mean serum globin levels were found to be high in patients with GI tract cancer on comparing their levels with those of non neoplastic GI tract disorders. The increase was found to be

statistically significant ($P < 0.01$) in above groups compared. The mean serum globulin levels were also found to be significantly ($P < 0.01$) high in patients with GI tract cancer on comparing their levels with those of patients suffering from liver cancer. Sherlock (1975) has also reported increased serum globulin values.

The albumin globulin ratio in normal controls, non malignant liver and GI tract disease controls and in liver and GI tract patients was found to be 2:1, 1:1.37, 1.45:1, 1:1.54, 1:1.44 respectively. The normal albumin globulin ratio reported by Varley (1980) is 1.5 - 2.0:1.

Albumin globulin ratio was found to be reversed in liver and GI tract cancer patients and also in non malignant liver disease controls and it has significantly decreased in non malignant GI tract disease controls on comparison with that of normal controls.

The alteration in the albumin globulin ratio is highly significant in liver disorders.

(ii) Serum total protein and gamma globulin levels

Table V shows the mean serum total protein, and gamma globulin content in normal controls, disease controls, and in liver and GI tract cancer patients,

TABLE-V

MEAN SERUM TOTAL PROTEIN AND GAMMAGLOBULIN CONTENT IN NORMAL CONTROLS DISEASE CONTROLS AND IN CANCER PATIENTS

Group	Type	No.	Total protein 9g/100ml) Mean ± S.D	Gamma Globulins (g/100ml) Mean ± S.D	Groups compared	't' Values
A	Normal controls	13	6,48 ± 0.59(A_1)	1.95 ± 0.32(A_2)	A_1 Vs B_1	2.243*
					A_1 Vs C_1	1.699NS
B	Non malignant liver disorders	11	6.92 ± 0.33(B_1)	3.76 ± 0.23(B_2)	A_1 Vs D_1	1.846NS
					A_1 Vs E_1	3.939**
C	Non malignant GI disorders	7	7.04 ± 0.79(C_1)	2.71 ± 0.62(C_2)	B_1 Vs D_1	4.005**
					C_1 Vs E_1	0.650NS
D	Liver cancer	9	5.96 ± 0.66(D_1)	3.36 ± 0.44(D_2)	D_1 Vs E_1	5.723**
					A_2 Vs B_2	14.982**
E	GI cancers	21	7.25 ± 0.49(E_1)	4.06 ± 0.39(E_2)	A_2 Vs C_2	3.430**
					A_2 Vs D_2	8.295**
					A_2 Vs E_2	17.160**
					B_2 Vs D_2	2.477*
					C_2 Vs E_2	6.502**
					D_2 Vs E_2	4.184**

** - $P < 0.01$

* - $P < 0.05$

NS - NOT SIGNIFICANT

From table V the mean total protein content of sera of normal controls, non malignant liver and GI tract disease controls, liver cancer and GI tract cancer patients is found to be 6.48 ± 8.59, 6.92 ± 0.33, 7.04 ± 0.79, 5.96 ± 0.66, 7.25 ± 0.49g/100ml respectively and the individual values are given in appendix-VIII and illustrated in figure-1.

The normal range of serum total protein level is 6.2-8.0g/100ml as reported by Nancy roper (1985). It is seen from the table V that the serum total protein value has increased above the normal controls value in disease conditions selected for the study except in liver cancer where the values were found to be lower than the normal value. But all the values are within the normal range as reported by Nancy roper, except that of liver cancer patients.

This increase in the serum total protein content was found to be statistically significant in GI tract cancer ($P < 0.01$) and in non malignant liver disorders ($P < 0.05$) when compared to that of the normal controls. However the increase noticed in GI tract non neoplastic disease is not statistically significant.

In liver cancer the serum total protein value was found to be lowered than that of the normal control, while a significant increase in the serum total protein value was recorded in non neoplastic liver disorders.

Although an increase in the serum total protein was noticed in both GI tract cancer and non neoplastic GI tract disorders on comparison with normal control, a significant difference in

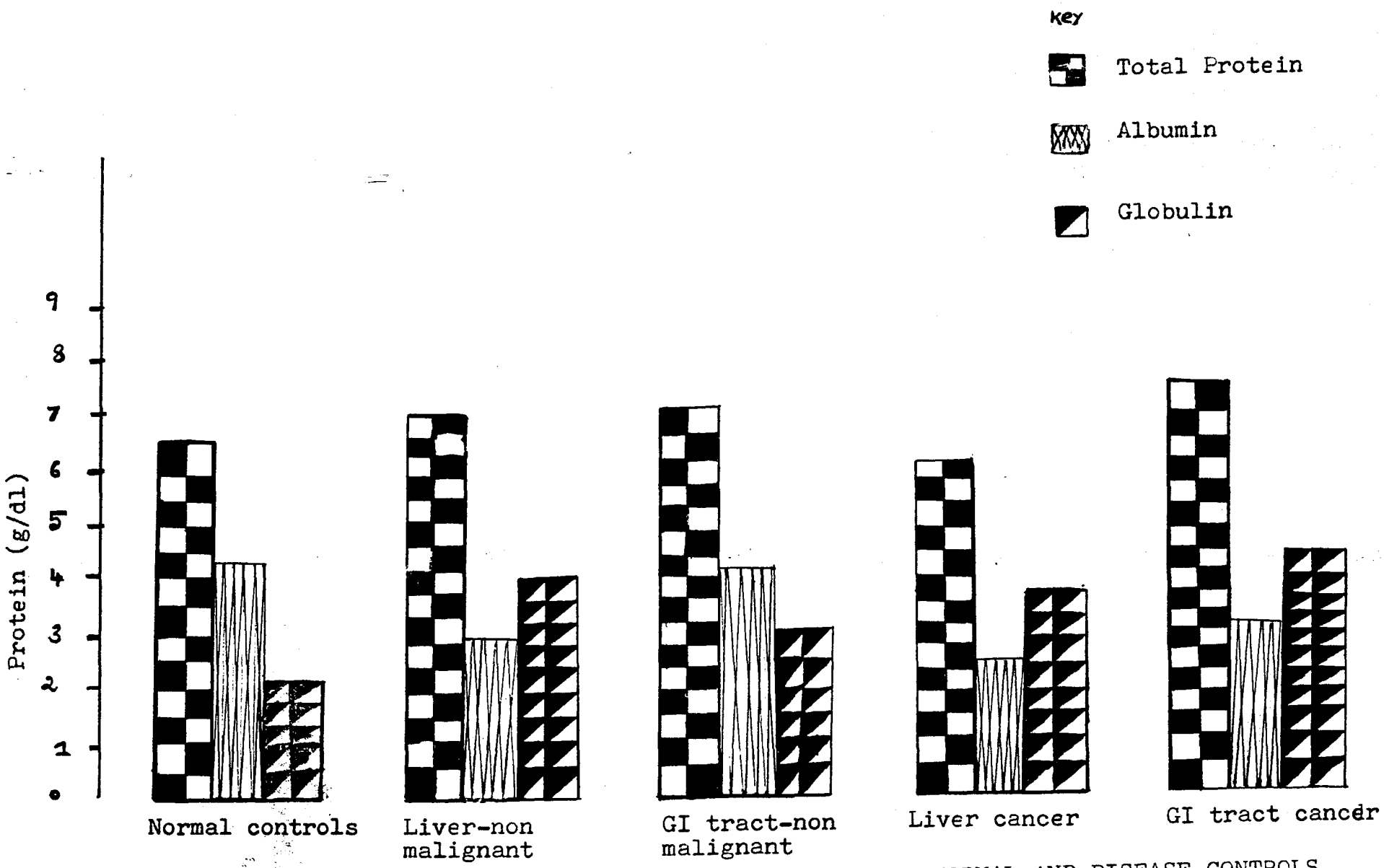


FIG-1 SERUM TOTAL PROTEIN, ALBUMIN, GLOBULIN LEVELS IN NORMAL AND DISEASE CONTROLS AND IN PATIENTS WITH CANCER

the value between these two groups was not seen.

While the patients with liver cancer showed a decrease in total protein value, the patients with GI tract cancer registered a significant increase compared to that of the normal controls.

Though there were alterations noticed in the differential protein fractions such as albumin, globulin, the total protein values were not altered significantly from the normal range. It may be due to the fact that the decreased albumin values were compensated by increased globulin fractions.

The mean serum gamma globulin levels 1.95 $\pm$ 0.32, 3.76 $\pm$ 0.23, 2.71 $\pm$ 0.62, 3.36 $\pm$ 0.44, 4.06 $\pm$ 0.39g/100ml in normal controls, liver and GI tract non neoplastic disease controls and liver and GI tract cancer patients are shown in table V and the individual values are given in appendix-VIII.

Nancy roper (1985) reported the normal range of serum gamma globulin level as 0.7 - 1.5g/100ml. The values were found to be significantly elevated ($P < 0.01$) in non neoplastic disease controls and cancer patients when compared to those of the normal controls.

The gamma globulin levels were found to be significantly higher in patients with non neoplastic diseases and cancer in the order of

GI tract cancer > Nonneoplastic liver disorders > Liver cancer > Non neoplastic GI tract disorders > Normal controls

(iii) Serum Immunoglobin levels

Table VI depicts the serum immunogloblins profile of normal controls, non neoplastic disease controls and cancer patients.

TABLE-VI

MEAN SERUM IMMUNOGLOBINS PROFILE OF NORMAL CONTROLS, DIASEASE CONTROLS AND IN CANCER PATIENTS

Group	Type	No.	IgG (mg/dl)		IgA (mg/dl)		IgM (mg/dl)	
			Range	Mean±S.D	Range	Mean±S.D	Range	Mean±s.D
A	Normal controls	2	2000-2050	2025±35.35	255.50-271.30	263.30±11.17	126.60-184.30	155.45±40.80
B	Non-neoplastic Liver disorders	4	2700-3160	2940±224.50	255.50-312.50	288.15± 2.39	176.65-216.07	194.41±17.36
C	Non-neoplastic GI tract disorders	2	2700-2860	2780±113.14	287.50-295.80	291.65± 5.87	176.65-192.05	184.35±10.89
D	Liver cancer	4	1643-3650	2585.75 ± 816.50	312.50-426.00	370.73±53.96	126.58-192.05	157.16±32.14
E.	GI tract cancer	6	2800-3650	3243.33 ± 344.77	295.80-530.00	358.30±86.38	199.93-440.00	331.30±106.6

As seen from table VI, the range of serum IgG levels of normal controls, liver and GI tract non neoplastic disorders, liver and GI tract cancer patients was found to be 2000-2050, 2700-3160, 2700-2860, 1643-3450, 2800-3600 mg/dl respectively and the individual values were given in appendix - VIII and illustrated in figure-2.

The normal range of IgG, IgA and IgM as reported by Hoechst (1986) is 712-1550, 120-220, 65-170mg/dl respectively. Based on this cirterion, the range of immunoglobulins of normal controls were higher than the reported value which may be attirbuted to the higher rate of infections and infestations in Indian population, Mahajan et al., 1985 have also reported similar findings. A wide range of IgG, IgA and IgM levels exist even in normal population.(Mahajan et al., 1985)

Since subsamples are used for immunoglobin estimation as shown in table, statistical analsysis (t-test) could not be done. The mean serum IgG levels were found to be elevated in all the cases taken for the study on comparison with that of normal controls. But the range of IgG levels shows a decrease in primary liver cancer when compared to normal controls. The mean serum IgG levels of patients with non neoplastic liver disease were found to be high on comparison with that of liver cancer patients. There was an increase in mean serum IgG levels of patients with GI tract cancer was noticed when compared to that of non neoplastic GI tract disease controls and liver cancer.

In a similar study, Malhotra et al. (1986) reported high

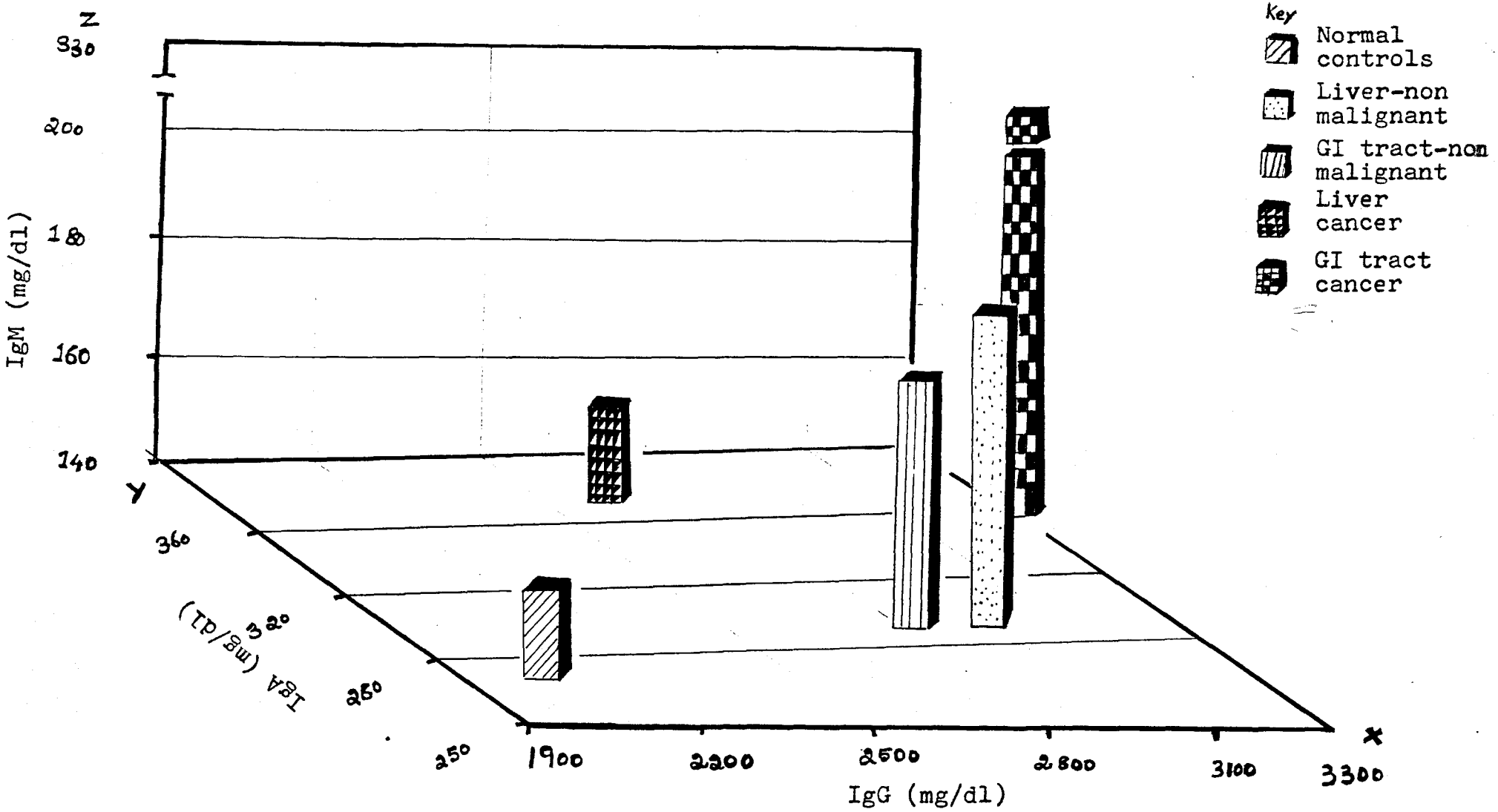


FIG-2 SERUM IMMUNOGLOBULINS PROFILE IN NORMAL AND DISEASED CONTROLS AND IN PATIENTS WITH CANCER

Three dimentional Bars:- Height of the Bars - IgM (mg/dl)
Position along X-axis IgG (mg/dl)
Position along Y-axis IgA (mg/dl)

immunoglobulins values in diverse hepatic disorders. Khandelwal and Khanna also reported low IgG values in primary liver cancer.

Circumstantial evidence in man suggests that immunity is important in host resistance against antigenic malignant disease (Kaur et al., 1986). Immunoglobulin levels found in patients may be related to antibody production capacity in malignancy as described by Kale et al. (1984).

Table VI gives the mean serum IgA levels of normal controls, liver and GI tract non neoplastic disease controls, liver and GI tract cancer patients, 263.30 ± 11.17, 288.15 ± 2.39, 291.65 ± 5.87, 370.73 ± 53.96, 358.3 ± 86.38mg/100ml respectively and the individual values are given in appendix - VIII and illustrated in figure-2.

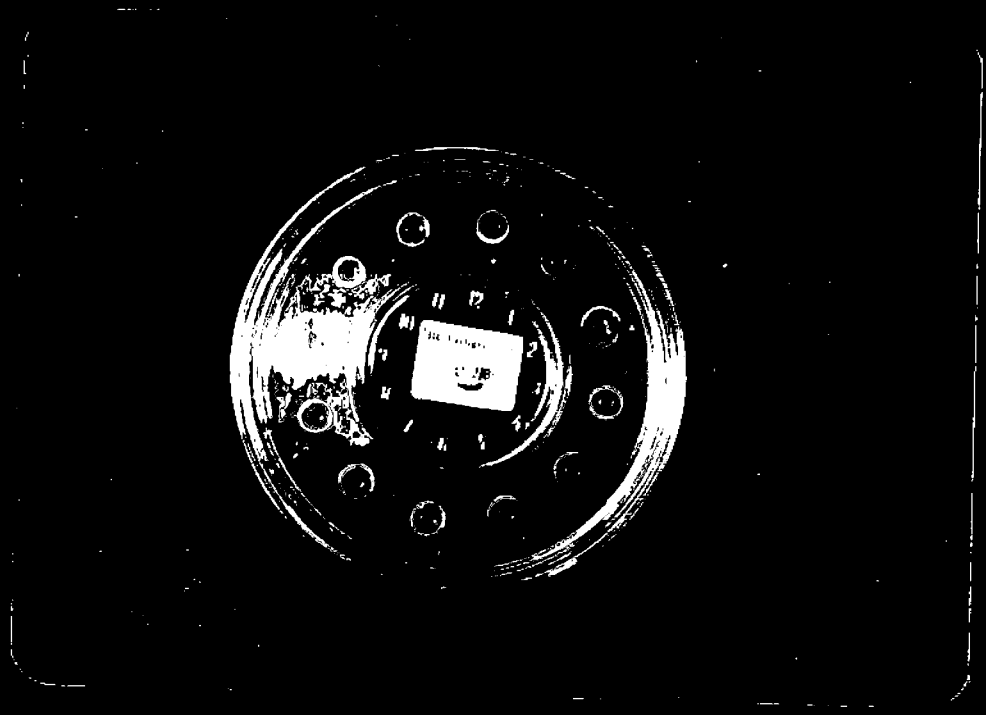
The mean serum IgA levels of the above said groups were found to be elevated when compared with normal controls, An increase in mean serum IgA levels was recorded in patients with liver cancer compared to that of patients with non neoplastic liver disorders. The difference in the mean serum IgA levels of GI tract cancer patients and GI tract non malignant diseases and also that of GI tract and liver cancer patients was found to be low. The increase in IgA levels was also reported in patients with liver cancer by Khandelwal and Khanna (1986) and Hughes (1971) in carcinoma of GI tract. Brown et al (1975), Wara et al (1977) and Cochran (1978) have also reported increased IgA levels in different malignancies.

IgA has a neutralising effect against viruses and antigentic stimulation of hepatitis B virus in the liver results in an increased synthesis of IgA with consequent increase in its concentration in the serum of liver cancer patients. Hughes (1971) suggested that increased IgA could be due to infection of the secreting mucosal epithelium as seen in carcinoma of the gut.

The results revealed the mean values of IgM in normal controls, non neoplastic liver and GI tract disease controls, liver and GI tract cancer patients were 155.45 ± 40.80, 194.41 ± 17.36, 184.35 ± 10.89, 157.16 ± 32.14, 331.30 ± 106.60mg per 100ml respectively and the individual values were given in appendix - VIII and illustrated in figure-2.

Eleveated means serum IgM levels were recorded in liver and GI tract non neoplastic disease controls, liver and GI tract cancer patientson comparing with normal controls. Increase in mean serum IgM levels was seen in patients with non neoplastic liver disease on comparing their levels with those of liver cancer patients. Increase in mean serum IgM levels was also noticed in patients with GI tract cancer on comparing their level with those of non malignant GI tract disorders. GI tract cancer patients showed high values of mean IgM levels than that of liver cancer patients.

The precipition rings formed in the tripartigen plates for IgG, IgA and IgM are shown in Plates I, II and III.



TRIPARTIGEN PLATE - IgG

TRIPARTIGEN PLATE - IgA

TRIPARTIGEN PLATE - IgM

(iv) Serum vitamin A level

Table VII depicts the mean serum vitamin A level of normal controls, non-neoplastic disease controls and cancer patients and the individual values are given in appendix-VII I

TABLE-VII

MEAN SERUM VITAMIN A LEVEL OF NORMAL CONTROLS, NON NEOPLASTIC DISEASE CONTROLS AND IN CANCER PATIENTS

Group	Type	No.	Serum Vitamin-A mcg/dl Mean±S.D	Groups compared	't' values
A	Normal controls	13	36.25±4.80	A Vs B	3.667**
				A Vs C	4.189**
B	Non neoplastic liver disorder	11	30.28±4.00	A Vs D	13.636**
C	Non-neoplastic GI tract disorder	7	27.82±2.00	A Vs E	13.852**
				B Vs D	12.044**
D	Liver Cancer	9	14.95±3.50	C Vs E	9.007**
E	GI tract cancer	21		D Vs E	2.136*

** - P< 0.01

* - P< 0.05

(iv) Serum vitamin A level

Table VII depicts the mean serum vitamin A level of normal controls, non-neoplastic disease controls and cancer patients and the individual values are given in appendix-VIII

TABLE-VII

MEAN SERUM VITAMIN A LEVEL OF NORMAL CONTROLS, NON NEOPLASTIC DISEASE CONTROLS AND IN CANCER PATIENTS

Group	Type	No.	Serum Vitamin-A mcg/dl Mean±S.D	Groups compared	't' values
A	Normal controls	13	36.25±4.80	A Vs B	3.667**
				A Vs C	4.189**
B	Non neoplastic liver disorder	11	30.28±4.00	A Vs D	13.636**
C	Non-neoplastic GI tract disorder	7	27.82±2.00	A Vs E	13.852**
				B Vs D	12.044**
D	Liver Cancer	9	14.95±3.50	C Vs E	9.007**
E	GI tract cancer	21		D Vs E	2.136*

** - $P < 0.01$

* - $P < 0.05$

As shown in table VII the mean serum vitamin A levels of the normal controls, non neoplastic liver disease controls, non neoplastic GI tract disease controls, liver cancer and GI tract cancer patients were found to be 36.25 ± 4.80, 30.29 ± 4.00, 27.82 ± 2.00, 12.27 ± 1.70 and 14.95 ± 3.50 microgram per 100ml and individual values are given in appendix-VIII and illustrated in figure-3.

It is evident that there was a remarkable reduction in the serum vitamin A in the different types of disease in question when compared to that of the normal controls.

Statistically significant ($P < 0.01$) difference in serum vitamin A was seen between cancer patients and the corresponding non neoplastic disease controls. Patients suffering from liver cancer exhibited a significantly ($P < 0.05$) lower serum vitamin A values than the GI tract cancer patients. Studies of Hiat et al., (1982), Schiffman (1983), Willet et al., (1984) and Joice et al., (1986) also have recorded such low values of serum vitamin A in cancer patients. The low levels of serum vitamin A in these patients might be due to low intake of food as a consequence of reduced or lack of appetite or might be the result of poor absorption as in the case of intestinal cancer.

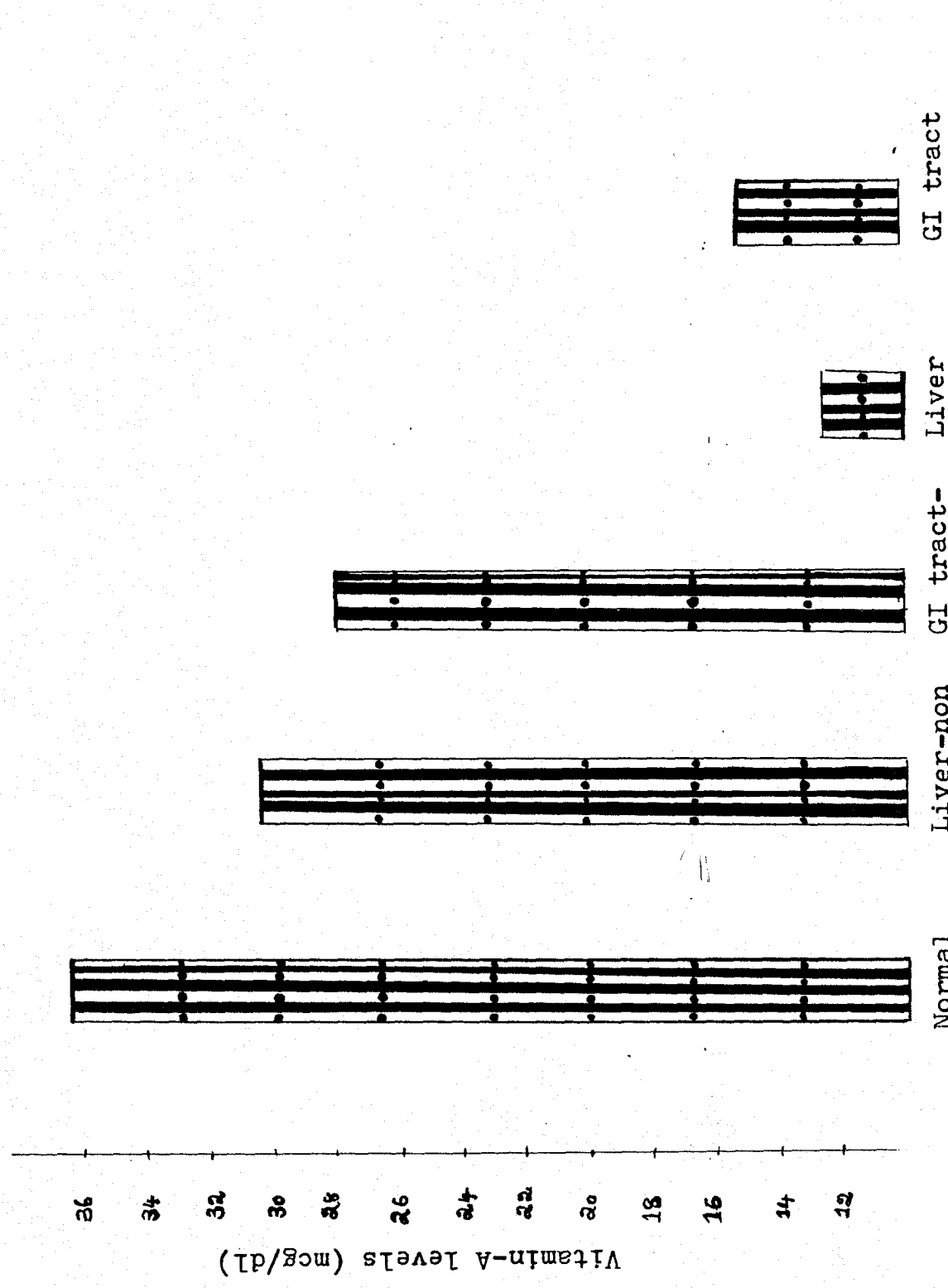


FIG-3 VITAMIN-A LEVELS IN NORMAL AND DISEASE CONTROLS AND IN PATIENTS WITH CANCER

(v) Serum zinc level

Table VIII presents the mean serum zinc levels of normal controls, non neoplastic disease controls and cancer patients and the individual values are given in appendix-VIII.

TABLE-VIII

MEAN SERUM ZINC LEVELS OF NORMAL CONTROLS, NON NEOPLASTIC DISEASE CONTROLS AND IN CANCER PATIENTS

Group	Type	No.	Serum zinc mcg/dl Mean ± S.D	Groups compared	't' values
A	Normal controls	13	84.20±7.09	A Vs B	1.576 NS
				A Vs C	3.418 **
B	Non neoplastic liver disorders	11	78.67±9.34	A Vs D	8.980 **
				A Vs E	10.625 **
C	Non neoplastic GI tract disorders	7	73.01±5.65	B Vs D	6.073 **
				C Vs E	4.844 **
D	Liver cancer	9	53.42±8.14	D Vs E	0.801 NS
E	GI tract cancer	21	49.85±11.76		

** - $P < 0.01$

* - $P < 0.05$

NS - NOT SIGNIFICANT

As seen from table VIII the serum zinc levels of normal controls liver and GI tract non neoplastic disease controls and liver and GI tract cancer patients were found to be 84.20 ± 7.09, 78.67 ± 9.34, 73.01 ± 5.65, 53.42 ± 8.14 and 49.85 ± 11.76mcg/100ml and the individual values are given in appendix-VIII and illustrated in figure-4.

In both liver and GI tract cancer patients and also in the corresponding non neoplastic disease controls a signicicant ($P < 0.01$) reduction in the serum zinc level was noticed when compared to that of the normal controls. The order of decrease in the serum zinc values in the different conditions chosen for the study was found to be as follows

Normal controls > Non neoplastic liver disease > Non neoplastic GI tract disases > Liver cancer > GI tract cancer

A significant difference ($P < 0.01$) was noticed between the mean serum zinc levels of cancer patients on one hand and those of the corresponding non neoplastic disease controls on the other. However, the difference between the mean serum zinc values of the liver and GI tract cancer patients was not found to be significant, low values of zinc in cancer patients have also been reported by Venketesan et al., (1983) and Giri et al., (1984).

Zinc is also shown to play a role in immune competence (Lindsey et al., 1986). So adequate intake of zinc may retard the tumor growth.

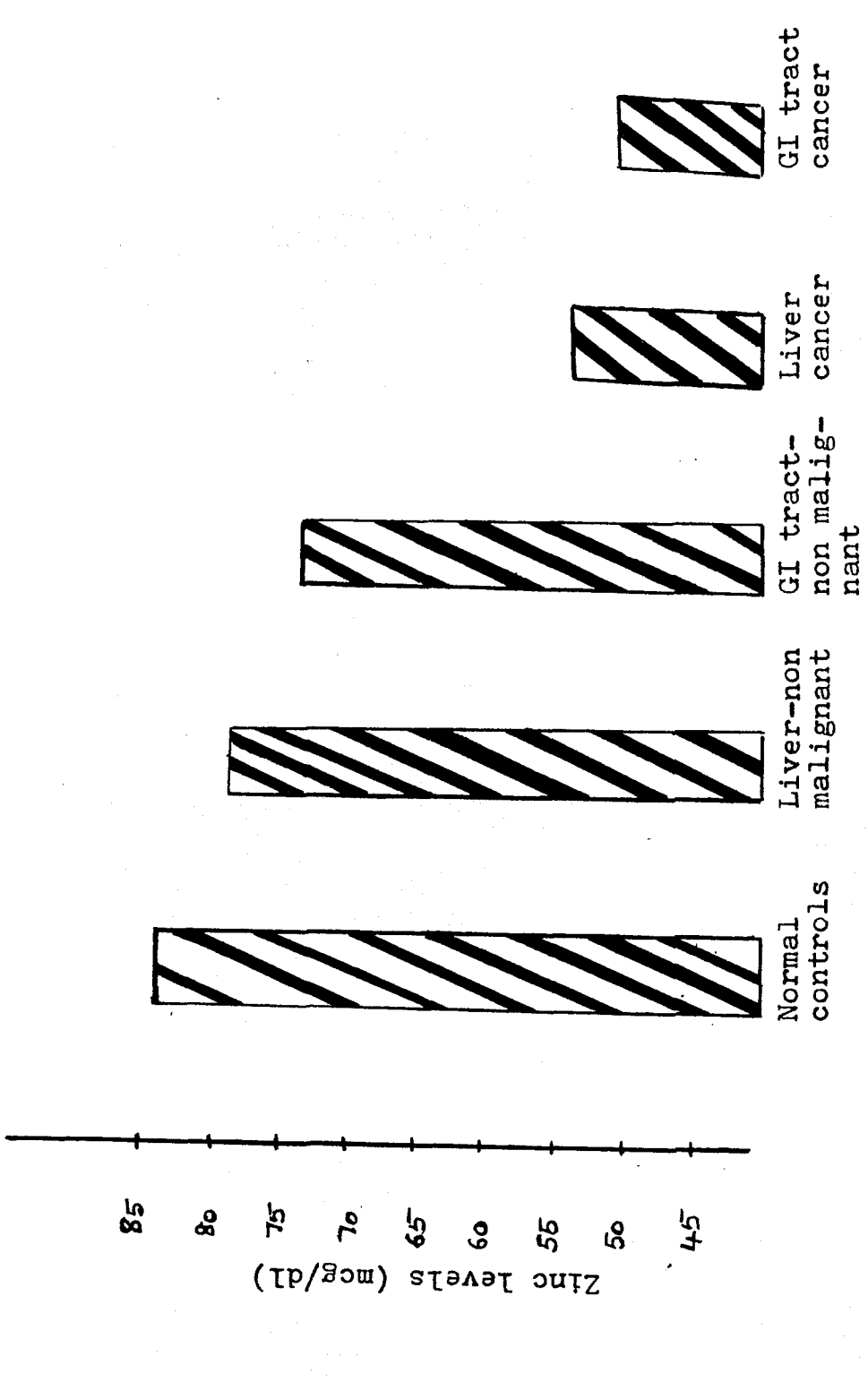


FIG-4 ZINC LEVELS IN NORMAL AND DISEASE CONTROLS AND IN PATIENTS WITH CANCER

The low serum zinc level observed in the patients might be due to the low intake of food as noticed in oesophagel and a stomach cancer and might be due to the poor absorption seen in intestinal cancer.

Hypoalbuminaemia has been suggested to be responsible for hypozincaemia (Venkatesan et al., 1983).

(vi) Total and differential count of leucocytes

(a) Total count and polymorphonuclear cells

Table IX gives the mean total leucocyte count, polymorphonuclear cells in normal controls, disease controls and cancer patients.

TABLE-IX

MEAN TOTAL LEUCOCYTE COUNT AND POLYMORPHONOCLEAR CELLS IN NORMAL CONTROLS, DISEASE CONTROLS AND IN CANCER PATIENTS

Group	Type	No.	Absolute number/cu.mm Mean ± S.D		Groups compared	't' values
			Total count	Polymorphonuclears		
A	Normal Controls	13	8030.80±769.60	5265.92±454.50	A_1 Vs B_1	1.684 NS
					A_1 Vs C_1	1.634 NS
B	Non malignant liver disorders	11	8845.53±1443.92	6055.40±1017.30	A_1 Vs D_1	5.087 **
					A_1 Vs E_1	6.352 **
					B_1 Vs D_1	2.780 *
C	Non malignant GI tract disorders	7	7471.43± 518.70	4863.00± 483.00	D_1 Vs E_1	7.066 **
					D_1 Vs E_1	0.151 NS
					A_2 Vs B_2	2.409 *
D	Liver Cancer	9	10894.40±1699.78	8096.23±1343.44	A_2 Vs C_2	1.754 NS
					A_2 Vs D_2	6.800 **
E	GI tract Cancer	21	10814.80±1754.30	7955.67±1468.12	A_2 Vs E_2	7.813 **
					B_2 Vs D_2	3.683 **
					C_2 Vs E_2	5.283 **
					D_2 Vs E_2	0.377 NS

** - $P < 0.01$

* - $P < 0.05$

NS - NOT SIGNIFICANT

From table IX the mean total leucocyte count of normal controls non neoplastic liver and GI tract disease controls and liver and GI tract cancer patients was found to be 8030.80 ± 769.60, 8845.53 ± 1443.92, 7471.43 ± 518.70, 10894.40 ± 1699.78, 10,814.80 ± 1754.30/cu mm and illustrated in figure-5.

It was seen that the mean total leucocyte count of liver and GI tract cancer patients were found to be significantly high ($P < 0.01$) on comparing with the leucocyte count of normal controls whereas the difference between the leucocyte count of liver and GI tract non malignant disease controls were found to be insignificant. Statistically significant ($P < 0.01$) difference in total leucocyte count was seen between cancer patients and the corresponding non neoplastic disease controls. No significant difference was noticed in total leucocyte count of liver and GI tract cancer patients. Sherlock (1975) and Kapoor et al., (1983) also have observed leucocytosis in cancer patients.

The mean polymorphonuclear cell count in normal controls, liver and GI tract non neoplastic disease controls and in liver and GI tract cancer patients was 5265.92 ± 454.50, 6055.40 ± 1017.30, 4863 ± 483, 8096 ± 1343.44, 7955.67 ± 1468.12/cu mm, as given in the table IX and individual values are given in appendix-VIII and illustrated in figure-5.

Thus polymorphonuclear cell count was found to be increased in all the conditions studied except in non malignant GI tract disorders compared to that of nomal controls. This increase in polymorphonuclear cell count was found to be statistically significant. The increase in the mean polymorphonuclear cell count seen in liver cancer patients was found to be statistically significant ($P < 0.01$) on comparing the values of non neoplastic liver disorders.

The increase in the mean polymorphonuclear cell count seen in GI tract cancer was also found to be statistically significant ($P < 0.01$) on comparing the values of non neoplastic GI tract diseases, but no significant difference was noticed when compared with the mean polymorphonuclear cell count of liver cancer.

(b) Lymphocytes and Eosinophils

Table X depicts the mean lymphocyte count and eosinophil count of normal controls, disease controls and cancer patients.

TABLE-X

MEAN LYMPHOCYTE AND EOSINOPHIL COUNT IN NORMAL CONTROLS, DISEASE CONTROLS AND IN CANCER PATIENTS

Group	Type	No	Absolute No/Cu.mm Mean ± S.D		Groups compared	't' values
			Lymphocytes	Eosinophils		
A	Normal Controls	13	2272.60±391.20 ($\bar{A}_1$)	400.77±191.90 ($\bar{A}_2$)	A_1 Vs B_1	3.827 **
					A_1 Vs C_1	6.137 **
B	Non neoplastic Liver disorders	11	1852.20±383.00 ($\bar{B}_1$)	870.00±399.70 ($\bar{B}_2$)	A_1 Vs D_1	6.170 **
					A_1 Vs E_1	4.101 **
					B_1 Vs D_1	1.735 NS
C	Non neoplastic GI tract disorders	7	1911.60±200.30 ($\bar{C}_1$)	654.60±231.40 ($\bar{C}_2$)	C_1 Vs E_1	1.255 NS
					D_1 Vs E_1	1.045 NS
D	Liver cancer	9	1525.89±417.50 ($\bar{D}_1$)	1176.67±368.20 ($\bar{D}_2$)	A_2 Vs B_2	3.591 **
					A_2 Vs C_2	2.485 *
E	GI tract cancer	21	1703.14±407.89 ($\bar{E}_1$)	1039.24±536.46 ($\bar{E}_2$)	A_2 Vs D_2	6.140 **
					A_2 Vs E_2	4.965 **
					B_2 Vs D_2	1.686 NS
					C_2 Vs E_2	1.776 NS
					D_2 Vs E_2	0.677 NS

** - $P < 0.01$
** -
* - $P < 0.05$

NS - NOT SIGNIFICANT.

From the table X the mean lymphocyte count of normal controls non neoplastic liver and GI tract disease controls, and liver and GI tract cancer was found to be 2272.60 + 391.20, 1852.20 ± 383.00, 1911.60 ± 200.30, 1525.89 ± 417.50, 1703.14 ± 407.89/cu mm and the individual values are given in appendix-VIII and illustrated in figure 5.

The mean lymphocyte count of the above said groups were found to be decreased on comparing the values of normal controls. This decrease in mean lymphocyte count was found to be statistically significant (P 0.01). But the difference in the mean lymphocyte count of cancer patients was found to be statistically insignificant on comparing these values with corresponding non neoplastic disease controls.

Lymphocytes are major carriers of cell mediated immune function that is why lymphocyte count is used as an index of cellular immune competence (Mahajan et al., 1985).

Depressed cell mediated immunity in various cancer has been observed by Haffejee et al., (1978), Burke et al., (1983) and Dickerson (1984). Similar results were noticed in the present study.

From the table X the mean eosinophil count of normal controls, non neoplastic liver and GI tract disease controls and liver and GI tract cancer was found to be 400.77 ± 191.90, 870.00 ± 399.70, 654.60 ± 231.40, 1176,67 ± 368.20, 1039.24 ± 536.46/c

and the individual values are given in appendix-VIII and illustrated in figure.5.

The mean eosinophil count of all the groups taken for the stdy showed a significant increase on comparing the mean eosinophil count of normal controls. The difference in the mean eosinophil count of cancer patients was found to be statistically insignificant on comparing these values with corresponding non neoplastic disease controls and also non significant difference was noticedin the mean eosinophil count of the two cancer in question.

Eosinophils are the part of the immunological reactions requiring the presence of antigens, macrophages and T lymphocytes. A progressive rise in the eosinophil count with advancement of cancer has been reported (Srivatsava et al., 1983).

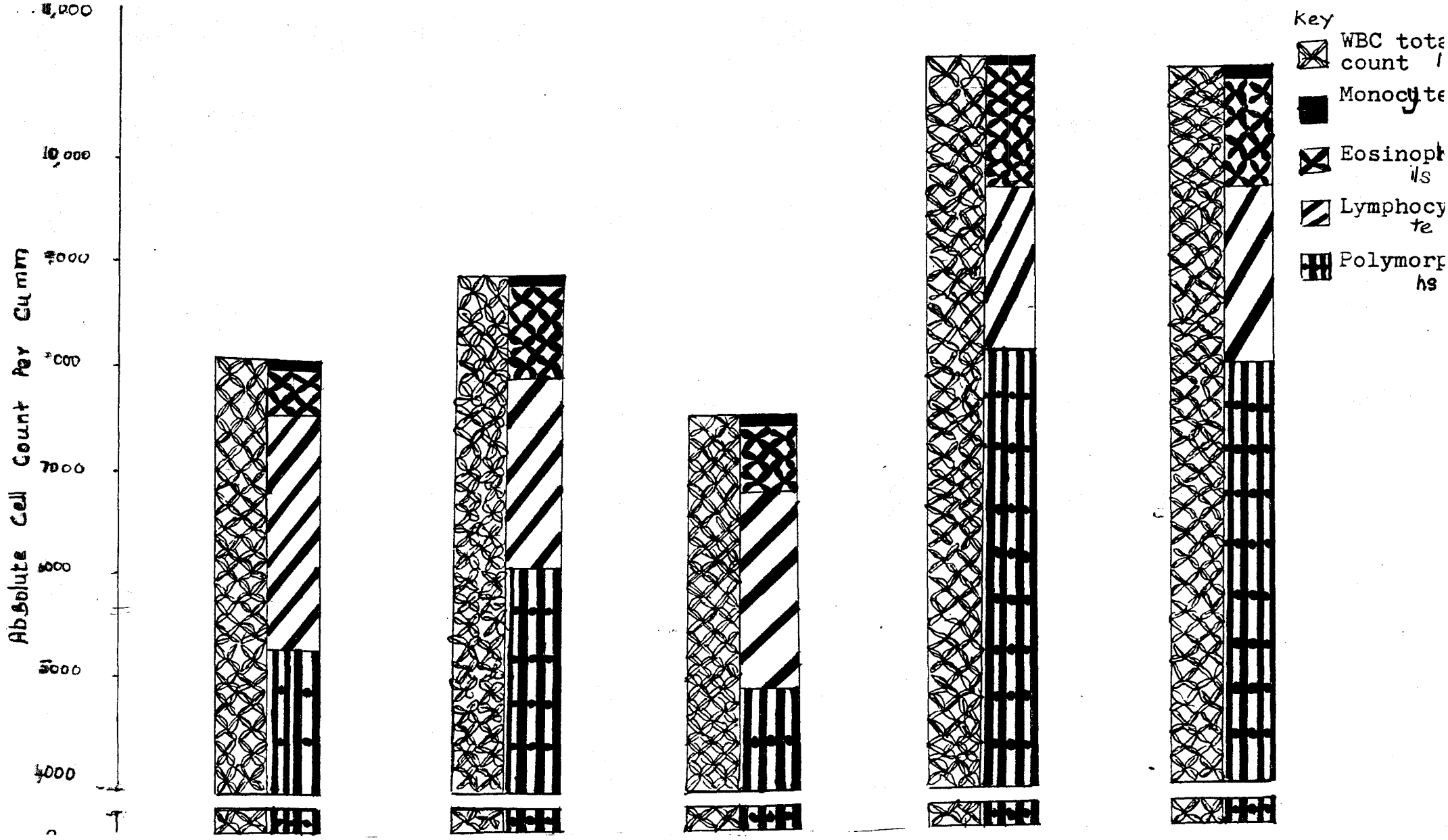


FIG-5 TOTAL AND DIFFERENTIAL COUNT OF LEUCOCYTES IN NORMAL AND DISEASE CONTROLS AND IN PATIENTS WITH CANCER

vii. Blood Haemoglobin

The mean blood haemoglobin values of the subjects selected for the study are presented in Table XI

TABLE-XI

MEAN BLOOD HAEMOGLOBIN VALUES OF NORMAL CONTROLS NON NEOPLASTIC CONTROLS AND IN CANCER PATIENTS

Group	Type	No.	Blood haemoglobin g/100ml Mean ± S.D	Groups compared	't' values	
A	Normal controls	13	12.87±1.08	A Vs B	7.842	**
				A Vs C	5.011	**
B	Non neoplastic liver disorders	11	8.60±1.47	A Vs D	7.133	**
				A Vs E	9.144	**
C	Non neoplastic GI tract disorders	7	10.02±1.27	B Vs D	0.441	NS
				C Vs E	2.122	*
				D Vs E	0.773	NS
D	Liver cancer	9	8.91±1.40			
E	GI tract cancer	21	8.45±1.74			

** - $P < 0.01$

* - $P < 0.05$

NS - NOT SIGNIFICANT

The mean blood haemoglobin value for the normal controls, non neoplastic liver disease controls, non neoplastic GI tract disease controls liver cancer and GI tract cancer patients was found to be 12.87 ±1.08, 8.60 ± 1.47, 10.02 ± 1.27, 8.91 ± 1.40 and 8.45 ± 1.74g/100ml of the blood respectively and the individual values are given in appendix-VIII and illustrated in figure- 6.

The blood haemoglobin concentration was found to be significantly ($P < 0.01$) lower in the non neoplastic disease controls and cancer patients chosen for this study when compared to that of normal controls. The difference in haemoglobin concentration between non neoplastic liver disease controls and liver cancer patients and that between the two types of cancer patients in question was found to be insignificant. On the other hand the difference in the haemoglobin concentration between non neoplastic GI tract disease controls and GI tract cancer patients was found to be statistically significant ($P < 0.05$). This suggested that haemoglobin concentration might play a role in the immune competance of the cancer patients.

The findings of this study are in accordance with the report of Kapoor et al., (1983) who found lower mean haemoglobin concentration among tumor bearing patients as compared to controls. The low levels of haemoglobin in the blood in both non-neoplastic disease and cancer of the GI tract might be due to the blood loss in the gut due to ulceration.

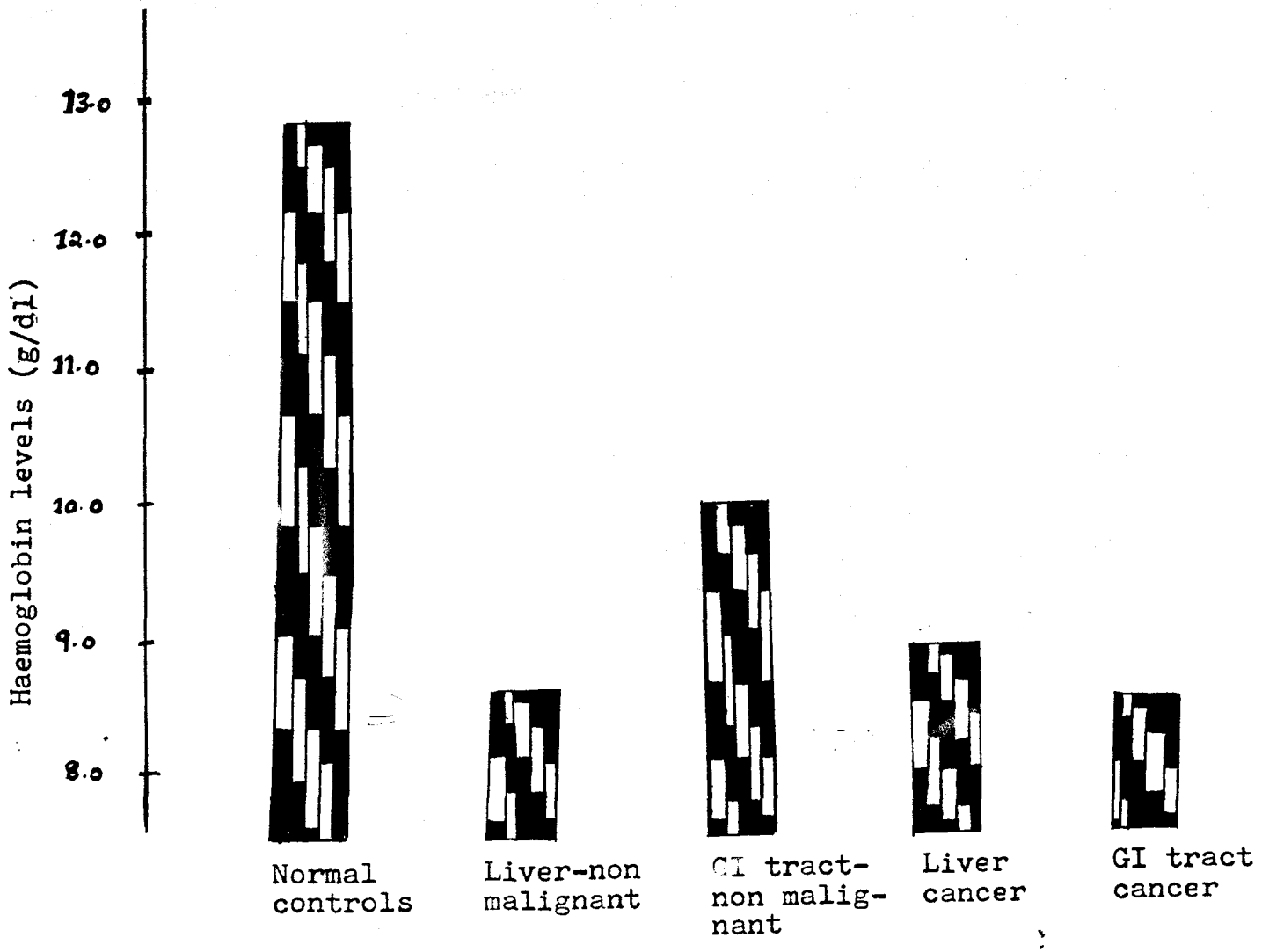


FIG-6 HAEMOGLOBIN LEVELS IN NORMAL AND DISEASE CONTROLS AND IN PATIENTS WITH CANCER

It has been well established in a series of hepotoma differentiated growth rates that there is a marked decrease in respiration and marked increase in glycolysis and so cancer cells intensively split glucose to lactic acid (Putul Maity et al.,1983 The above altered metabolism may be due to decreased haemoglobin levels which might have decreased oxygen supply to the cells.

VIII, Lysozyme Activity in Plasma

The mean plasma lysozyme activity in the selected normal controls, non neoplastic disease controls and cancer patients is shown in Table XII.

TABLE-XII

MEAN PLASMA LYSOZYME ACTIVITY IN NORMAL CONTROLS NON NEOPLASTIC DISEASE CONTROLS AND CANCER PATIENTS

Group	Type	No.	Plasma lysozyme activity(mcg/ml) Mean±S.D	Groups compared	't' values
A	Normal controls	13	9.93±1.05	A Vs B	8.000**
				A Vs C	7.500**
B	Non neoplastic liver disorder	11	15.45±2.08	A Vs D	5.100**
C	Non neoplastic GI tract disorder	7	13.13±0.29	A Vs E	9.500**
				B Vs E	2.800*
D	Liver Cancer	9	12.96±1.63	C Vs E	1.100NS
E	GI tract cancer	21	13.63±1.19	D Vs E	1.200NS

** - $P < 0.01$

* - $P < 0.05$

Table XII gives the mean lysozyme activity in normal controls, non neoplatic liver disease controls, non neoplastic GI tract disease controls liver cancer and GI tract cancer patients was found to be 9.93 ± 1.05, 15,45 ± 2.08, 13.13 ± 0.29, 12.96 ± 1.63 and 13.63 ± 1.19mcg per ml respectively and the individual values are given in appendix-VIII and illustrated in figure.7.

Thus plasma lysozyme activity was found to be increased in all the desease conditions studied compared to that found in normal controls. This elevation in plasma lysozyme activity was found to be statistically significant (P 0.01) and this elevation in the different disease conditions was found to be in the following order of magnitude.

Normal controls < Liver cancer < Non neoplastic GI tract disease < GI tract cancer < Non neoplastic liver disease

This finding is in agreement with Miale (1982) who also observed a high concentration of lysozyme in serum of almost all cases of acute monocytic leukemia.

Lysozyme is a nonspecific antibacterial substance present in almost all the tissues and fluids in the body (Stryer1981). So the increase in lysozyme activity may not only be due to cancer but also due to the bacterial infections.

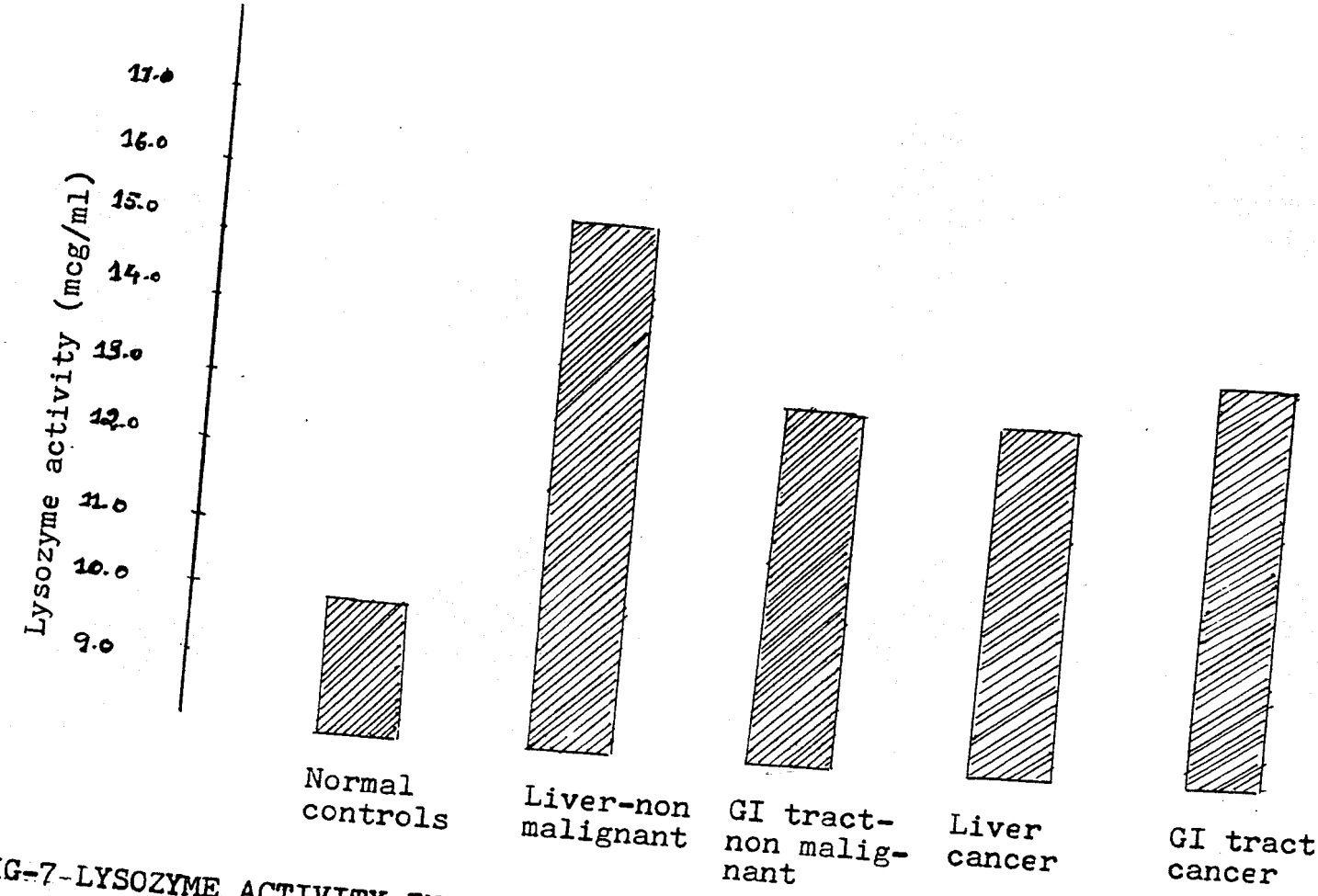


FIG-7-LYSOZYME ACTIVITY IN NORMAL AND DISEASE CONTROLS AND IN PATIENTS WITH CANCER

D. Comparison of primary and secondary cancers of liver

Table XIII projects the biochemical profile of primary and secondary liver cancer patients.

TABLE - XIII

BIOCHEMICAL PROFILE OF FIVE PRIMARY AND FOUR SECONDARY LIVER CANCER PATIENTS

No.	Parameters	Primary liver cancer Mean±S.D	Secondary liver cancer Mean±S.D	't'values
1.	Total protein (g/dl)	5.44±0.18	6.60±0.32	5.573**
2.	Albumin (g/dl)	2.29±0.25	2.58±0.18	1.004NS
3.	Globulin (g/dl)	3.25±0.09	4.03±0.21	6.016**
4.	Gamma globulin (g/dl)	3.01±0.09	3.81±0.18	7.029**
5.	IgG (mg/dl)	1921.50±393.86	3250.00±282.80	-
6.	IgA (mg/dl)	369.25± 80.26	372.20± 47.80	-
7.	IgM (mg/dl)	162.70± 41.49	151.60± 35.40	-
8.	Vitamin A (mcg/dl)	12.25± 1.66	12.42± 1.99	0.116NS
9.	Zinc (mcg/dl)	52.52± 6.30	54.55± 10.99	0.550NS
10.	Leucocyte total count (/cu.mm)	10440.00±2052	11462.50±1145.60	0.767NS
11.	Polymorphs (/cu.mm)	8010.80±1664	8203.00±1045.20	0.713NS
12.	Lymphocyte (/cu.mm)	1404.00± 235.80	1678.25± 578.75	0.512NS
13.	Eosinophils (cu.mm)	991.00± 293.00	1408.75± 342.98	1.633NS
14.	Haemoglobin (g/dl)	8 8.32± 1.40	9.66± 1.22	1.273NS
15.	Lysozyme activity (mcg/ml)	13.12± 1.74	12.75± 0.30	0.370NS

** $P < 0.01$

NS-NOT SIGNIFICANT

As shown in table XIII, comparison of various biochemical parameters in primary and secondary liver cancer revealed a significant ($P < 0.01$) difference between the mean values of total protein, globulin, gamma globulins and IgG in primary and secondary liver cancer patients. IgG level was found to be high in secondary liver cancer.

E. Comparison of different types of the gastrointestinal cancer

(a) Stomach and oesophagealcancer

Table XIV depicts the biochemical profile of stomach and oesophageal cancer patients.

TABLE-XIV

BIOCHEMICAL PROFILE OF FOUR STOMACH AND ELEVEN OESOPHAGEAL CANCER PATIENTS

No.	Parameters	Mean ± S.D		't'values
		Stomach cancer	Oesophageal cancer	
1.	Total protein (g/dl)	7.25±0.39	7.23±0.51	0.066NS
2.	Albumin (g/dl)	2.77± 0.42	3.05± 0.41	1.082NS
3.	Globulin (g/dl)	4.48± 0.14	4.18± 0.34	1.595NS
4.	Gamma globulin(g/dl)	4.44± 0.19	3.99± 0.31	2.968**
5.	IgG (mg/dl)	3550.00± 141.42	3155.00± 417.23	-
6.	IgA (mg/dl)	412.90± 165.60	329.75± 12.23	-
7.	IgM (mg/dl)	256.75± 80.35	355.60± 161.79	-
8.	Vitamin-A (mcg/dl)	11.78± 2.15	15.83± 3.92	0.475NS
9.	Zinc (mcg/dl)	60.15± 8.08	48.43± 13.26	1.545NS
10.	Total leucocyte(/cu.mm)	10677.50±1269.39	11200.00±2223.00	0.414NS
11.	Polymorphs(/cu.mm)	7812.75±1400.77	8182.09±1833.74	0.219NS
12.	Lymphocyte(/cu.mm)	1757.25± 140.29	1754.00± 537.90	0.011NS
13.	Eosinophils(/cu.mm)	944.25± 238.69	1155.36± 619.40	0.618NS
14.	Lysozyme (mcg/dl)	14.00± 1.42	13.89± 1.19	0.140NS
15.	Haemoglobin(g/dl)	8.36± 2.50	8.24± 1.59	0.102NS

** - $P < 0.01$

NS - NON SIGNIFICANT

As in clear from Table XIV comparison of various biochemical parameters in oesophageal and stomach cancer showed no significant difference.

(b) Stomach and Intestinal cancer

Biochemical profile of four stomach and six intestinal cancer patients are given in table XV.

TABLE-XV

BIOCHEMICAL PROFILE OF FOUR STOMACH AND SIX INTESTINAL CANCER PATIENTS

No.	Parameters	Stomach cancer Mean±S.D	Intestinal cancer Mean±S.D	't'values
1.	Total protein (g/dl)	7.25±0 0.39	7.29± 0.58	0.125NS
2.	Albumin (g/dl)	2.77± 0.42	3.04± 0.21	1.260NS
3.	Globulin (g/dl)	4.48± 0.14	4.25± 0.43	1.114NS
4.	Gamma globulin(g/dl)	4.44± 0.19	4.08± 0.47	1.561NS
5.	IgG (mg/dl)	3550.00± 141.42	3025.00±318.20	-
6.	IgA (mg/dl)	412.90± 165.60	332.30± 27.90	-
7.	IgM (mg/dl)	256.75± 80.35	381.58± 82.62	-
8.	Vitamin A (mcg/dl)	11.78± 2.15	15.45± 2.09	2.681*
9.	Zinc (mcg/dl)	60.15± 8.08	45.57± 7.17	2.957**
10.	Total leucocyte (/cu.mm)	10677.50±1269.39	10200.00±812.00	0.694NS
11.	Polymorphs(/cu.mm)	7812.75±1400.77	7635.80± 68.30	0.276NS
12.	Lymphocyte(/cu.mm)	1757.25± 140.29	1573.80±216.50	1.558NS
13.	Eosinophils(/cu.mm)	944.25± 238.69	889.67±534.77	0.205NS
14.	Hemoglobin (g/dl)	8.36± 2.50	8.89± 1.72	0.383NS
15.	Lysozyme activity mcg/ml)	14.00± 1.42	12.90± 0.84	1.460NS

** - $P < 0.01$

* - $P < 0.05$

NS - NOT SIGNIFICANT

It is clear from the above table that only IgG, IgM, vitamin A and zinc levels showed significant difference between the two types of cancer considered.

(c) Oesophageal and Intestinal cancer

Table XVI shows the biochemical profile of oesphageal and intestinal cancer patients.

TABLE-XVI

COMPARISON BETWEEN ELEVEN OESOPHAGEAL AND SIX INTESTINAL CANCER

No.	Parameters	Oesophageal cancer Mean ± S.D	Intestinal cancer Mean ± S.D	't' value
1.	Total protein (g/dl)	7.23 ± 0.51	7.29 ± 0.58	0.224 NS
2.	Albumin (g/dl)	3.05 ± 0.41	3.04 ± 0.21	0.054 NS
3.	Globulin (g/dl)	4.18 ± 0.34	4.25 ± 0.43	0.377 NS
4.	Gamma Globulin (g/dl)	3.91 ± 0.31	4.08 ± 0.47	0.906 NS
5.	IgG (mg/dl)	3155.00 ± 417.23	3025.00 ± 318.20	
6.	IgA (mg/dl)	329.75 ± 12.23	332.30 ± 27.90	
7.	IgM (mg/dl)	355.60 ± 161.79	381.58 ± 82.62	
8.	Vitamin A (mcg/dl)	15.83 ± 3.92	15.45 ± 2.09	0.056 NS
9.	Zinc (mcg/dl)	48.43 ± 13.26	45.57 ± 7.17	0.473 NS
10.	Total Leucocyte (/cu.mm)	11200.00 ±2223.00	10200.00 ± 812.00	0.934 NS
11.	Polymorphs (/cu.mm)	8182.09 ±1833.74	7635.80 ± 68.30	0.687 NS
12.	Lymphocyte (/cu.mm)	1754.00 ± 537.90	1573.80 ± 216.50	0.751 NS
13.	Eosinophils (/cu.mm)	1155.36 ± 619.40	889.67 ± 534.77	0.877 NS
14.	Hemoglobin (g/dl)	8.24 ± 1.59	8.89 ± 1.72	0.789 NS
15.	Lysozyme activity (mcg/ml)	13.89 ± 1.18	12.90 ± 0.84	1.770 NS

Table XVI revealed that there was no significant difference between intestinal and oesophageal cancer patients in the various biochemical parameters studied.

It is now generally accepted that competant host defense mechanism can be an important determinant response to neoplastic disease. The human malignancies contain specific antigens and that the major part of the host's response against a tumor involves the delayed hypersensitivity immune reaction mediated by T cell lympocytes (Brookes and Clifford, 1981).

Evidence that cancer patients may have impaired immune responsiveness that could influence the course of their diseases gives importance to studies of immunologic competance in such patients (Nawalka et al., 1983)

An attempt was made in the present study to determine the antibody mediated and cell mediated immune reactions (in terms of immunoglobulins and lymphocytes count respectively) in cancer patients in response to cancer. The results showed depressed lymphocyte count and increased serum immunoglobulin levels. The parameters which affect the immune profile such as zinc, vitamin A in serum, Haemoglobin levels were also found to be decreased. Low serum albumin levels, high plasma lysozyme activity and leucocytosis were also noticed. These results showed the involvment of immune reactions in cancer.

In the present study only thirty cancer patients were studied. Decisions cannot be made with the analysis of only few samples. Due to the inadequate facilities, the levels of specific antigens and the monoclonal antibodies specific for each cancer could not be studied. Analysis of above factors with more samples may throw more light in making a determination of immune profile in cancer patients with respect to immunotherapy.

Along with controls, diseased controls (Non malignant liver and GI disorders) were also taken for comparison. It was seen that some of the biochemical changes were qualitatively similar in cancerous and non cancerous disorders of liver and GI tract.

Cirrhosis of liverwas described as a precancerous stage (Khandelwal and Khanana, 1986). Oesophagitis (leiman et al., 1986) and chronic gastric ulcer (Manohar, 1984) were reported as the risk factors for oesophageal and stomach cancer respectively. More studies regarding these precancerous stages may help to decrease the incidence of cancer by preventing them in the very early stages.

Summary and Conclusion

SUMMARY AND CONCLUSIONS

The present study is of a pioneering kind and enabled an understanding of changes in immune profile in liver and gastrointestinal cancer. The investigation consisted of measuring the serum total protein, albumin, globulin, gamma globulin, immunoglobulin fractions, vitamin A, zinc and total and differential count of leucocytes, heamoglobin and plasma lysozyme activity. An attempt was made to see whether the analysis of these constituents in cancer patients can throw some light to establish the relationship between the immune profile and cancer.

Thirty liver and gastrointestinal cancer patients who were undergoing treatment during January-February, 1987 in Coimbatore Medical College Hospital, Coimbatore, Kuppuswamy Naidu Memorial Hospital, Pappanaickenpalayam and Employees State Insurance Hospital, Singanallur were selected for the study. They were in the age group of 30-70 years and of both sexes. Thirteen normal healthy individuals of the same age group and another group of who were suffering from liver and gastrointestinal non neo plastic disorders served as normal controls and diseased controls respectively for comparison.

1. The mean serum albumin level of normal controls was found to be 4.30 ± 0.31g/dl, in non neo plastic liver and GI tract disease controls, it was 2.94 ± 0.27 and 4.06 ± 0.23g/dl, respectively in neo plastic liver and GI tract disease it was 2.36 ± 0.26, 2.99 ± 0.36g/dl. The mean serum albumin level of non-malignant liver disorder, liver cancer and GI tract cancer was found to be significantly low ($P < 0.01$) compared with that

of normal controls but the change in albumin level seen in GI tract, non neoplastic disease controls was not statistically significant. Liver cancer patients showed a statistically significant lower value of mean serum albumin than the mean serum albumin levels of GI tract cancer patients ($P<0.05$) and non malingnant liver disease controls ($P<0.01$). The low albumin value found in GI tract cancer patients was found to be significant ($P<0.01$) when compared with that of patients with non-neoplastic GI disorders. Albumin is synthesized in the liver In severe hepatocellular disease, the level of albumin falls. Inflammation of the liver stimulates the synthesis of a number of plasma proteins and in doing so appear to divert the protein synthetic apparatus of the hepatocyte away from synthesis of albumin. For this reason plasma albumin levels fall in all chronic diseases (West, 1985).

2. The mean serum globulin content of normal controls, non neoplastic liver and GI tract disease controls, liver and GI tract cancer was 2.18 ± 0.33, 3.98 ± 0.26, 2.98 ± 0.66, 3.59 ± 0.43, 4.26 ± 0.35g/dl. Hyper-globulinemia was noticed in non malignant liver disease controls as well as in cancer patients, whereas in GI tract disease controls it was found to be within the normal range on comparison with that of normal controls. The difference in globulin content of the different patients was found to be statistically significant when compared with that of the normal controls and also that of different groups of patients. A significant increase in serum globulin levels was observed in patients with non malignant liver disease on comparing their levels with those of the liver cancer patients. The mean serum

globulin levels were found to be high in patients with GI tract cancer on comparing their levels with those of non malignant GI tract disorders. The increase was found to be statistically significant ($P < 0.01$) in above groups. The mean serum globulin levels were also found to be significantly ($P < 0.01$) high in patients with GI tract cancer when compared with those of patients suffering from liver cancer.

3. The albumin globulin ratio in normal controls non malignant liver and GI tract disease controls, liver and GI tract cancer was found to be 2:1, 1: 1.37, 1.45:1, 1:1.54, 1:1.44 respectively. Albumin globulin ratio was found to be reversed in liver and GI tract cancer patients and also in non neoplastic liver disease controls and it has significantly decreased in non neoplastic GI tract desease controls on comparison with that of normal controls.

4. The mean total protein content of sera of normal controls, patients with non malignant disorders of liver and GI tract, liver and GI tract cancer was found to be 6.48 ± 8.59, 6.92 ± 0.33, 7.04 ± 0.79, 5.96 ± 0.66, 7.25 ± 0.49g/100ml. The serum total protein value was found to be increased above the value of the normal controls, in disease conditions, selected for the study, except in liver cancer, where the values were found to be lower than that of the normal controls.

This increase in the serum total protein content was found to be statistically significant in GI tract cancer ($P < 0.01$)

and in non malignant liver disorders ($P < 0.05$) when compared to that of the normal controls. However the increase noticed in GI tract non neoplastic disease is not statistically significant. In liver cancer the serum total protein value was found to be lowered than that of the normal control while a significant increase in serum total protein value was recorded in non neoplastic liver disorders. Although an increase in the serum total protein was noticed in both GI tract cancer and non neoplastic GI tract disorders on comparison with normal control, a significant difference in the value between these two groups was not seen. Though there was alterations noticed in the differential protein fractions such as albumin, globulin, the total protein values were not altered significantly from the normal range as reported by Nancy ropper (1985) (Normal range 6.2-8.0g/dl). It may be due to the fact that the decreased albumin values were compensated by high levels of globulins.

5. The mean serum gamma globulin levels were 1.95 ± 0.32, 3.76 ± 0.23, 2.71 ± 0.62, 3.36 ± 0.44, 4.06 ± 0.39g/100ml in normal controls liver and GI tract non malignant disease controls, liver and GI tract cancer patients reapectively.

The values were found to be significantly elevated ($P < 0.01$) in non neoplastic disease controls and cancer patients when compared with those of the normal controls.

6. The range of serum IgG levels of normal controls, liver and GI tract non neoplastic disorders, liver and GI tract cancer patients was found to be 2000-2050, 2700-3160, 2700-2860, 1643-3450, 2800-3600mg/dl respectively.

The normal range of IgG, IgA and IgM reported by Hoechst (1986) is 712-1500, 120-220, 65-170mg/dl respectively. Based on this criterion, the range of immunoglobulins of normal controls were higher than the reported value which may be attributed to the higher rate of infections and infestations in Indian population, A wide range of IgG, IgA and IgM levels exist even in normal population. Since subsamples are used for immunoglobulin estimation, statistical analysis (t-test) could not be done. The mean serum IgG levels were found to be elevated in all the cases taken for the study on comparison with that of normal controls. But the range of IgG levels shows a decrease in primary liver cancer when compared to normal controls. The mean serum IgG levels of patients with non neoplastic liver disease were found to be high on comparison with that of liver cancer patients. An increase in mean serum IgG levels of pateints with GI tract cancer was noticed when compared to that of non neoplastic GI tract disease controls and liver cancer.

In a similar study, Malhotra et al. (1986) reported high immunoglobulins values in diverse hepatic disorders. Khandalwal and Khanna (1986) also reported low IgG values in Primary liver cancer.

Circumstantial evidence in man suggests that immunity is important in host resistance against antigenic malignant disease (Kaur et al., 1986). Immunoglobulin levels found in patients may be related to antibody production capacity in malignancy as described by Kale et al., (1984).

The mean serum IgA levels of normal controls, liver and GI tract non neoplastic disease controls, liver and GI tract cancer patients 263.3 ± 11.17, 288.15 ± 2.39, 291.65 ± 5.87, 370.73 ± 53.96, 358.3 ± 86.38mg/100ml respectively, ~~and the individual values are given in appendix-VIII.~~ The mean serum levels of the above said groups was found to be elevated when compared with normal controls. An increase in mean serum IgA levels was recorded in patients with liver cancer compared to that of patients with non neoplastic liver disorders. The difference in the mean serum IgA levels of GI tract cancer patients and GI tract non malignant diseases and also that of GI tract and liver cancer patients was found to be low.

IgA has a neutralising effect against viruses and antigenic stimulation of hepatitis B virus in the liver results in an increased synthesis of IgA with consequent increase in its concentration in the serum of liver cancer patients. Huges (1971) suggested that increased IgA could be due to infection of the secreting mucosal epithelium as seen in carcinoma of gut.

The mean values of IgM in normal controls, non neoplastic liver and GI tract disease controls, liver and GI tract

cancer patients were 155.45 ± 40.80, 194.41 ± 17.36, 184.35 ± 10.89, 157.16 ± 32.14, 331.30 ± 106,6mg/100ml respectively.

Elevated mean serum IgM levels were recorded in liver and GI tract non neoplastic disease controls, liver and GI tract cancer patients on comparing with normal controls. Increase in mean serum IgM levels was deen in patients with non neoplastic liver disease on comparing their levels with those of liver cancer patients. Increase in mean serum IgM levels was also noticed in patients with GI tract cancer on comparing their levels with those of non malignant GI tract disorders. GI tract cancer patients showed high values of mean serum IgM levels than liver cancer patient.

7.(a) The mean leucocyte count of normal controls, non neoplastic liver and GI tract disease controls, liver and GI tract cancer patients was found to be 8030.80 ± 769.60, 8845.53 ± 1443.92, 7471.43 ± 518.70, 10894.40 ± 1699.78, 10814.80 ± 1754.3/cu mm. Leucocytosis was noticed in cancer patients ($P < 0.01$) on comparing with normal controls whereas the difference between the leucocyte count of liver and GI tract non malignant disease controls were found to be insignificant. Statistically significant ($P < 0.01$) difference in total leucocyte count was seen between cancer patients and the corresponding non neoplastic disease controls. No significant difference was noticed in total leucocyte count of liver and GI tract cancer patients.

(b) The mean polymorphonuclear cell count in normal controls, patients with liver and GI tract disorders, liver and GI tract

cancer patients was found to be 5265.92 ± 454.50, 6055.40 ± 1017.30, 4863 ± 483.00, 8096.23 ± 1343.44, 7955.67 ± 1468.12/cu mm. Thus polymorphonuclear cell count was found to be increased in all the conditions studied except in non malignant GI tract disorders compared to that of normal controls. The increase in the mean polymorphoneclear cell count seen in liver cancer patients was found to be statistically significant ($P < 0.01$) on comparing the values on non neoplastic liver disorders.

The increase in the mean polymorphonuclear cell count seen in GI tract cancer was also found to be statistically significant ($P < 0.01$) on comparing the values of non neoplastic GI tract diseases, but no significant difference was noticed when compared with the mean polymorphonuclear cell count of liver cancer.

(c) The mean lymphocyte count of normal controls, non neoplastic liver and GI tract disease controls, liver and GI tract cancer was found to be 2272.60 ± 391.20, 1852 ± 383.00, 1911.60 ± 200.30, 1525.89 ± 417.50, 1703.14 ± 407.89/cu mm.

The mean lymphocyte count of the above said groups was found to be decreased from that of the normal controls. This decrease in mean lymphocyte count was found to be significant ($P < 0.01$). But the difference in the mean lymphocyte count of cancer patients was found to be statistically insignificant when compared with that of the non neoplastic disease controls. Lymphocytes are major carriers of cell mediated immune function

that is why lymphocyte count is used as an index of cellular immune competence, (Mahajan et al., 1985).

(d) The mean Eosinophil count of liver and GI tract cancer patients was found to be 400.77 ± 191.90, 870.00 ± 399.70, 654.60 ± 231.40, 1176.67 ± 368.20, 1039.24 ± 536.46/cu mm.

The mean eosinophil count of all groups taken for the study showed a significant increase on comparing the mean eosinophil count of normal controls.

The difference in the mean eosinophil count of cancer patients was found to be statistically insignificant on comparing these values with corresponding non neoplastic disease controls and also no significant difference was noticed in the mean eosinophil count of the two cancers in question. This revealed that eosinophils are part of the immunological reactions requiring the presence of antigens, macrophages and T lymphocytes.

8. The mean serum vitamin A levels of the normal controls, non neoplastic liver disease controls, non neoplastic GI tract disease controls, liver and GI tract cancers were found to be 36.25 ± 4.80, 30.28 ± 4.00, 27.82 ± 2.00, 12.27 ± 1.70 and 14.95 ± 3.50 mcg/100ml. It is evident that there was a remarkable reduction in the serum vitamin A in the different types of disease in question when compared to the normal controls. Statistically significant ($P < 0.01$) difference inserum vitamin A was seen between cancer patients and

corresponding non neoplastic disease controls. Patients suffering from liver cancer exhibited a significantly low serum vitamin A values than the GI tract cancer patients. The low levels of serum vitamin A in those patients might be due to low intake of food as a consequence of reduced or lack of appetite or might be the result of poor absorption as in the case of intestinal cancer.

9. The mean serum zinc levels of normal controls, liver and GI tract non neoplastic disease controls, liver and GI tract cancer patients was found to be 84.20, 78.67, 73.01,53.42 and 44.85mcg/100ml. In both liver and GI tract cancer patients and also in corresponding non neoplastic disease controls a significant ($P < 0.01$) reduction in the serum zinc was noticed when compared to that of normal controls.

A significant difference ($P < 0.01$) was noticed between the mean serum zinc levels of cancer patients on one hand amd those of the corresponding non neoplastic disease controls on the other. However the difference between the mean serum zinc values of the liver and GI tract cancer patients was not found to be significant. Zinc is shown to play a role in immune competence (Lindsey et al., 1986). So adequate intake of zinc may retard the tumor growth. The low serum zinc level observed in patients might be due to the low intake of food as noticed in oesophageal and stomach cancers and might be due to the poor absorption seen in intestinal cancer. Hypoalbuminaemia has been suggested to be responsible for hypozincaemia. (Venkatesan et al., 1983)

10. The mean heamoglobin value for the normal controls, non neoplastic liver disease controls, non neoplastic GI tract disease controls, liver and GI tract cancer patients was found to be 12.87 ± 1.08, 8.60 ± 1.47, 10.02 ± 1.27, 8.91 ± 1.40 and 8.45 ± 1.74g/100ml of the blood respectively. The blood heomoglobin concentration was found to be significantly ($P < 0.01$) lower in the above groups chosen for the study when compared with normal controls. The difference in heamoglobin values between neoplastic group was found to be insignificant on the other hand the difference in haemoglobin concentration between non neoplasic GI tract disease controls and GI tract cancer patients was found to be statistically significant ($P < 0.05$). This suggested that haemoglobin concentration might play a role in the immune competence of the cancer patients. It has been well established in a series of hepatoma differentiated growth rates that there is a marked increase in glycolysis and so cancer cells intensively split glucose to lactic acid (Putul Maity et al., 1983) The above altered metabolism may be due to decreased heamoglobin levels which might have decreased oxygen supply to the cells.

11. The mean lysozyme activity in normal controls, non neoplastic liver and GI tract controls, liver and GI tract cancer patients was found to be 9.93 ± 1.05, 15.45 ± 2.08, 13.13 ± 0.29, 12.96 ± 1.63 and 13.63 ± 1.19mcg/dl. Thus plasma lysozyme activity was found to be increased in all the disease conditions studied compared to that found in normal controls. This elevation in plasma lysozyme activity was found to be statistically significant

($P < 0.01$). Lysozyme is a non specific antibacterial substance present in almost all the tissues and fluids in the body (Stryer 1981). So the increase in lysozyme activity may be not only due to the cancer but also due to the bacterial infections.

12. Comparison of various biochemical parameters in primary and secondary liver cancer revealed a significant ($P < 0.01$) difference between the mean values of total proteins, globulin, gamma globulins and IgG in primary and secondary liver cancer patients. IgG level was found to be high in secondary liver cancer.

13. Comparison of various biochemical parameters in oesophageal and stomach cancer, oesophageal and intestinal cancer showed no significant difference. Only IgG, IgM, vitamin A and zinc levels showed difference between the stomach and intestinal cancers.

The results showed leucocytosis, depressed lysmphocyte count, increased serum immunoglobulin fractions and increased plasma lysozyme activity. The parameters which affect the immune profile such as zinc, vitamin A in serum, haemoglobulin levels were also found to be decreased and low serum albumin levels were also noticed.

It is generally accepted that competence host defense mechanism can be an impartant reaponse to neoplastic disease, since human malignancies contain specific antigens and that the major part of the host response against a tumor involves the delayed hypersensitivity immune reaction mediated by T cell lymphocytes (Brookes and Clifford, 1981).

Recommendations

In the present study which is of a pilot kind, only 30 samples had been analysed. Decisions cannot be made with the analysis of a few samples. Due to inadequate facilities the levels of specific antigens and the monoclonal antibodies specific for each cancer could not be studied. Analysis of above factors with more samples may throw more light in making a determination of immuneprofile in cancer patients with respect to immunotherapy. Monoclonal antibodies directed against human tumor associated antigens or marker can potentially be used as carriers of radio activity for in vivo diagnosis and treatment of solid tumors (Buchegger et al., 1986).

The idea to link a toxic agent to a monoclonal antibody binding to a particular tumor antigen, thus fashioning a magic bullet that will destroy targeted cancer cells but leave normal cells unharmed (Collier and Kaplan, 1984). The available evidence also suggests that if such a treatment gets success, it may lessen the problem of non specific toxicity hitherto a major impediment to development of more effective cancer treatment, especially for metastatic disease.

It is suggested that an investigation can be undertaken to find out whether a massive doses of vitamin A to cancer patients would arrest the malignancy, as vitamin A might promote the immune response.

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Appendices

APPENDIX - I

ESTIMATION OF SERUM TOTAL PROTEIN, ALBUMIN, GLOBULIN AND GAMMA GLOBULIN BY BIURET METHOD

Principle

The colorimetric method for protein estimation makes use of biuret reaction. Substances which contain - $CoNH_2$ group joined directly or through a single carbon or nitrogen atom give a blue purple colour with alkaline copper sulphate solution. Proteins thus give a purple colour which is different for different proteins. The reaction takes its name from the complex formed i.e., biuret.

Reagents

1) Stock biuret reagent

Dissolved 45g of rochelle salt in about 400ml of 0.2 N sodium hydroxide and 15g of copper sulphate $5H_2O$ stirring continuously until the solution was complete. Added 5.0g of potassium iodide and made upto a litre with 0.2N sodium hydroxide.

2) Dilute biuret reagent

Diluted 200ml of stock reagent to a litre with 0.2N sodium hydroide which contains 5.0g of potassium iodide per litre.

3)Standard protein solution

Weighed 400mg of albumin and dissolved in 0.9% sodium chloride solution so that 1.0ml of this solution contains 4.0mg of protein.

4) 0.9% Saline.

5) 22.5% Sodium sulphate solution.

6) Ammonium sulphate - Sodium chloride reagent (Wolfson et al., 1948).

Dissolved 193 g of ammonium sulphate in about 500ml water added 40 g sodium chloride, dissolved and made upto a litre. Kept at room temperature.

Procedure

Into a series of test tubes pipetted out 0.5 ml - 2.5 ml of standard protein solution. The volume was then made upto 2.5 ml with water. Into another tube added 0.1 ml of serum and diluted to 2.5 ml with 0.9%saline. Now added 3.0 ml of dilute biuret reagent to all the tubes. Along with this blank was also taken. The colour developed was read at 500 millimicron colorimetrically after 30 minutes.

A standard graph was drawn by plotting concentration on X-axis and colorimeter reading on Y-axis. The amount of protein present was calculated. This gives the total protein value for serum.

Precipitation of Globulin

Globulin was precipitated by mixing 0.2 ml of serum with 4.8 ml of 22.5% sodium sulphate. Stoppered the tubes and left in the incubator at 40° C overnight. Filtered the solution next day using Whatman Number 42 filter paper. Took 2.5 ml of filtrate and carried out the experiment as for the rest. The concentration of gram percentage of albumin present in globulin free filtrate was determined from the graph.

Precipitation of gamma globulins

Measured 4.8 ml of Ammonium sulphate - sodium chloride reagent into a 15 ml tapered centrifuge tube, layered 0.2 ml serum on top and mixed by slow, careful inversion until turbidity was maximal. Stoppered the tube and centrifuged for 30 minutes at 2500 rpm. (If the supernatant fluid is still not quite clear, cool in running water and centrifuge again) For accurate results the supernatant fluid must be crystal clear. Carefully poured off most of this without disturbing the precipitate centifuged again for few minutes, carefully inverted and stood on a filter paper to drain as completely as possible. Added 2.0 ml water, shook vigorously and carried out the experiment as for the rest.

APPENDIX - II

QUANTITATIVE DETERMINATION OF SERUM IMMUNOGLOBULINS IgG, IgA AND IgM, USING TRI-PARTIGEN IMMUNODIFFUSION PLATES (BEHRIBGWERKE, 1986).

Composition

Tripartigen immunodiffusion plates contain a prepared agar gel in which H - chain specific anti serum to the respective immunoglobulin is incorporated. The antiserum is produced by immunization of sheep and goats.

Preservatives

Sodium azide (1.0 mg/ml)

Sodium p - ethyl - mercury - mercaptobenzene.

Sulphonate (at most 0.1 mg/ml).

Method

Tripartigen is suitable for Quantitative immunoglobulin determination (using reference values supplied with each plate)

(1) Opened the plate and left the opened plate to stand for about 5 minutes at room temperature to allow any condensation water that may have accumylated in the wells to evaporate.

(2) IgA and IgM were determined using undiluted serum. The sera tested and the control serum were diluted 1:10 with isotonic saline for the determination of IgG.

(3) Procedure

Well 1 was filled with 5 mcl of control serum.

Wells 2-12 were each filled with 5 mcl of the respective sera under test.

(4) Closed the plate tightly and left it to stand at room tempetature. Evaluation was made after a minimum diffusion time of 50 hours (IgG and IgA and 80 hours IgM).

(5) At the end of the given diffusion time, the diameter D of the precipitation rings was measured accurately to 0.1 mm using a suitable calibrated instrument.

(6) Evaluation

The immunoglobulin concentrations related to the measured diameters were read directly from the table of reference values. When determining IgG, the value found was multiplied by the dilution factor.

If protein concentrations of the serum samples diverse considerably from normal value, this means that the resulting precipitin ring diameters will fall outside the assay range of plate, In this case the examination must be repeated, using higher or lower dilutions of the serum sample.

TABLE OF REFERENCE VALUES

Diameter	IgG	IgA	IgM
	Values in mg/dl		
4.o	16.60	21.40	39.48
4.1	18.90	25.30	44.43
4.2	21.20	29.40	49.51
4.3	23.50	33.50	54.70
4.4	25.97	37.80	60.02
4.5	28.50	42.10	65.46
4.6	31.00	46.50	71.02
4.7	33.60	51.10	76.71
4.8	36.30	55.70	82.51
4.9	38.96	60.40	88.44
5.0	41.70	65.30	94.49
5.1	44.55	70.20	100.67
5.2	47.40	75.20	106.96
5.3	50.40	80.30	113.38
5.4	53.40	85.60	119.92
5.5	56.40	90.90	126.58
5.6	59.50	96.30	133.37
5.7	62.70	101.80	140.28
5.8	65.90	107.40	147.31
5.9	69.10	113.10	154.46

contd...

6.0	72.50	118.90	161.73
6.1	75.80	124.80	169.13
6.2	79.30	130.80	176.65
6.3	82.80	136.90	184.29
6.4	86.30	143.10	192.05
6.5	89.90	149.40	199.93
6.6	93.60	155.80	207.94
6.7	97.30	162.30	216.07
6.8	101.10	168.90	224.32
6.9	104.90	175.60	232.70
7.0	108.80	182.30	241.19
7.1	112.70	189.20	249.81
7.2	116.70	196.20	258.55
7.3	120.80	203.30	267.42
7.4	124.80	210.40	276.40
7.5	129.00	217.70	285.51
7.6	133.20	225.10	294.74
7.7	134.50	232.50	304.09
7.8	141.80	240.10	313.56
7.9	146.20	247.80	323.16
8.0	150.70	255.50	332.88
8.1	155.20	263.40	342.72
8.2	159.70	271.30	352.68
8.3	164.30	279.40	362.77
8.4	169.00	287.50	372.98
8.5	173.70	295.80	383.31
8.6	178.50	304.10	393.76
8.7	183.30	312.50	404.33
8.8	188.20	321.10	415.03

APPENDIX - III

ESTIMATION OF SERUM VITAMIN A (RETINOL).

The serum vitamin A was estimated by the trifluoroacetic acid method of Neeld and Pearson as modified and suggested by Roels et al., quoted by Gyorgy and Pearson (1967).

Reagents

1. Absolute ethanol: Purified for spectrophotometry,
2. n-hexane: Fisher certified reagent special for spectrophotometry.
3. Chloroform: Merck reagent special for spectrophotometry.
4. Trifluoroacetic acid Reagent grade (Sigma).
5. 1N alcoholic potassium hydroxide.
6. Stock vitamin A

344 mg of vitamin A acetate (300 mg of vitamin A) was dissolved in chloroform and made upto 100ml. 1.0 ml of stock contains 3000 mcg of retinol.

Intermediate standard

1. 0.1 ml of stock standard diluted to 100 ml with chloroform (3 mcg/ml)
2. 0.1 ml of stock diluted to 50 ml with chloroform (6 mcg/ml)
3. 0.15 ml of stock diluted to 50 ml with chloroform (9mcg/ml)
4. 0.1 ml of stock diluted to 25 ml with chloroform (12 mcg/ml)

C. Working Standard

Each intermediate standard was again diluted in the ratio

1:100 and from each standard finally 1.0 ml was taken.

Technique

The serum (0.5 ml or less) was saponified with an equal volume of 1 N ethanolic potassium hydroxide in a waterbath at 60°c for 20 minutes. The mixture was cooled and vigorously shaken in a glass stoppered tube with an equal volume (1.0 ml) of n-hexane for 10 minutes. The tube was centrifuged for 1 minute at 100 g to seperate the layers. An aliquot (0.8ml) was pipetted off for the determination of retinol. The n-hexane was evaporated from this aliquot in a waterbath 60° C in a steam of oxygen free nitrogen. The last traces of n-hexane were removed by nitrogen blowing at room temperature. The residue was taken up in (0.5 ml) chloroform, shaken vigorously and the optical density at 620 nm was determined exactly 30 seconds after addition of the trifluoroacetic acid.

Correction of vitamin A Transmission reading at 620 nm for carotene interference at that wavelength.

Trifluoroacetic acid in chloroform solution reacts with carotene, and the absorption of the reaction product at 620 nm, interferes with the viramin A determination done at the same wavelength. A series of the solution of the beta carotene standards in petroleum ether were made and aliquot portions of it were evaporated and the residue was taken up in chloroform and optical density was read at 452 nm.

A second series of solutions of the beta carotene standard in petroleum ether was made, aliquot portions of it were evaporated from centrifuge tubes and exactly the same procedure was applied to the residues remaining in the centrifuge tubes as was described for serum. The optical density at 620nm contributed by the beta carotene TFA complex was subtracted from the total optical density at 620 nm, and true value of the optical density due to vitamin A was employed in the final calculation.

APPENDIX - IV

ESTIMATION OF SERUM ZINC

For the estimation of zinc Piper's method (1966) was followed.

Principle

Serum on digestion with triple acid (nitric acid, sulphuric acid and perchloric acid) in the ratio (9:2:1) liberates into solution the trace elements.

Technique

1.0ml of the serum sample was taken in a microkjeldhal digestion flask which was previously washed with glass distilled water. It was dried and to this was added 10.0ml of triple acid. The mixture was shaken and digested on a sand bath with occasional shaking. The digestion was continued till no more brown fumes evolved and the solution in the flask became colourless. The digested mixture was transferred to a 25ml standard flask, the washing being done with double distilled water. This solution was used for analysing Zinc, using the atomic absorption spectophotometer (AA 120 model) available in Soil Science Department in Tamil Nadu Agricultural University, Coimbatore.

PROCEDURE FOR ROUTINE ANALYSIS

(1) Selected the lamps to be used and inserted them in the lamp Quadrants.

(2) Depressed the relevant LAMP-SELECT button for the lamp being used and set the METER SELECT to the same lamp.

(3) Switched on the instrument. Set the lamp at the desired current and allowed to stabilise for 10-15 minutes.

(4) Set the indicator unit in the TRANSMISSION mode with the select switch in 'NORMAL'.

(5) Set the monochromator to the wave length required with the relevant slit opening and using again setting to give approximately 80% T reading.

(6) Selected the desired mode of operation on the indicator unit i.e., ABSORBANCE OF TRANSMISSION.

(7) Selected the 'AUTO 100' mode and trimmed the 'SET 100' to read 0.0 Absorbance or 100% transmission.

(8) Lighted the flame.

(9) Nebulised the sample into the flame.

APPENDIX - V

ESTIMATION OF WHITE BLOOD CELLS

For the estimation of white blood cells Truck's fluid method and Leismann stain method were followed (Samuel, 1966).

Apparatus

Microscope, WBC Pipette, Neubauer ruling.

Diluting Fluid

This is composed of gention violet to stain the nucleus and acetic acid to destroy the RBC and distilled water for dilution.

Procedure

Cleaned the finger tip with spirit. Allowed it to dry. Pricked the finger with the surgical needle. A sudden prick was given so that a free flow of blood was obtained. Drew the blood upto 0.5 mark and diluted upto the mark. The pipette was held firmly by its ends between the four fingers and thumb and rotated so that the content was mixed well. The mixture was then applied to the narrow slit between the counting chamber and the cover slip. The fluid runs under the cover slip. The slide was set aside for 2 minutes for the cells to set.

Counting

Counting was done under a high power objective. Each medium sized square contains 16 small squares. The cells were counted from 5 sets of 16 small squares. The squares were

counted simultaneously in horizontal rows of 4 at a time.

Differential count of Leucocytes

Procedure

Blood stains can be made either on slides (glass) or cover glasses. To get a thin uniform blood smear, the slide must be free from grease. Hence the slides were cleaned with glacial acetic acid followed by water and alcohol. Three such slides were taken, one was used as a spreader and the other two were covered with blood film. The ends of the spreader were pinched off to make the film narrower than the slide.

Preparation of Blood smear

Cleaned the finger with spirit. Allowed it to dry and pricked it with a clean needle to obtain a drop of blood. Applied one end of the slide to the drop of blood. Placed the slide on some smooth surface and held it in position with the thumb and the index finger of the left hand. The narrow edge of the spreader was placed in the drop and held there until the blood had spread across it. It was then drawn slowly over the whole length of the first slide. The inclination of the spreader should be 45° and hence it was held between thumb index and middle finger with their tips resting on the table to avoid undue pressure. Allowed the film to dry in air.

Staining the film by Leishman's Stain

This is a simplification of the method of staining introduced by Romanowsky. The stain consists of a mixture of methylene blue and eosin in methyl alcohol. The dry film was covered with this stain for one minute. Double the quantity of distilled water was added to the stain so as to stay on the slide without spilling. Mixed it thoroughly with rocking movement. The diluted stain was allowed to stain for six minutes. The mixture was then poured off. This was then covered with distilled water and washed with it until the pink colour appeared. Allowed it to dry in oil. Covered the smear with cidar wood oil and examined it under a microscope using oil immersion objective.

Estimation of the film

Methyl alcohol acts acts as a fixative. In a properly stained film the RBC's are pink, the cytoplasm of the white cell blue, small neutrophil granules purple, large eosinophil granules brick red and basophil granules dark blue.

A differential count can be carried out efficiently using a mechanical stage and oil immersion objective. The differential count is done to ascertain the relative number of different varieties of leucocytes. Counted atleast a hundred leucocytes and tabulated in a column, using the mechanical

stage travelled the full width of the film, moved the film a little and examined in the reverse direction, This avoids repetition of counting the same fluid. A stained film also shows morphological characteristics of red and white cells. From the percentage value obtained, absolute number is calculated.

APPENDIX - VI

ESTIMATION OF HAEMOGLOBIN BY CYANMETHAEMOGLOBIN METHOD

(Cart Wright, 1958).

Estimation of haemoglobin by this method is recommended by Xth international Haematology Congress and World Health Organisation Expert Committee on nutritional Anaemias.

This method measures not only oxyhaemoglobin but also carbonmonoxide haemoglobin and methemoglobin except sulphaemoglobin. With filter type photo electric colorimeters the single relatively broad band of cyanmethaemoglobin in the green spectral region, has a distinct advantage.

This method can be modified to determine haemoglobin in dry blood on filter paper also.

Reagents

Drabkin's Diluent Solution

Sodium Bicarbonate 1 g.

Potassium Cyanide 0.05 gm.

Potassium Ferricyanide 0.20 gm.

Distilled water to make 1 litre

This solution should not be used after it forms a precipitate on the bottom of the storage bottle. The solution was preferably under cold storage. Its preparation and handling should be done with great care.

Procedure

1. Exactly 5.0 ml of Drabkins diluent solutions was measured into a dry test tube from pipette.

2. Exactly 0.02 ml of blood was transferred from a standardized Haemoglobin pipette into the diluent solution. Usual care in filling and cleaning of loaded haemoglobin pipette must be observed.

3. The pipette was rinsed three times with the diluent solution without allowing the formation of air bubbles in the solution.

4. The blood and the diluent were thoroughly mixed by rotating the tube.

5. 10 minutes time was allowed for the formation of the cyanomethemoglobin.

6. 5.0 ml of the diluent solution was used as blank.

7. With green filter No:540 the readings were taken in a photo electric colorimeter.

Calibration procedure

1. Total blood iron was determined by Wong's method. This determination gives absolute amount of Haemoglobin .

2. Exactly 0.02 ml of this known blood was measured as above into 5.0, 7.5, 12.5, 15.0 ml respectively of

diluent solution and mixed by rotating the tubes.

These solutions are now equivalent to blood samples containing respectively 100, 67, 50, 40 and 30% that of the original solution.

3. The intensity of the colour was read using green filter 540 against diluent as a blank set at zero O.D.

4. On a graph paper a standard graph was drawn using these haemoglobin concentration and corresponding density values.

APPENDIX - VII

ESTIMATION OF LYSOZYME CONTENT OF PLASMA(Harrison, 1968)

The assay was based on the lysis of Micrococcus Lysodeikticus cells by lysozyme. The varient used was as follows.

Phosphate buffer 0.067 M, pH 6.2 was used for the substrate and standards. The substrate was freshly prepared suspension of dried Micrococcus Lysodeikticus cells. (obtained as a free gift from sigma chemical company, California) 25 mg/100ml.

The stock standard contained 400 mcg/ml and was kept at 5°C. Working standards freshly prepared contained 0.4, 0.8, 2, 4, 10, 20 mcg/ml. Measurements were made at 450 nm in a Beckmann Du spectrophotometer using 2 cm cells with the slit adjusted, so that the extinction of the substrate was 0.65.

0.5 ml standard plasmawas added to 5.0 ml of substrate and the mixture was kept at 25° C. Extinctions were measured at 30 seconds ($E\frac{1}{2}$) and 10 minutes (E10) and $E\frac{1}{2}$-E10 was calculated in each case.

A standard curve was constructed with each batch of estimations. Enzyme concentration in the samples was related to the crystalline egg white standard without allowing for the different specific activities of human and egg white lysozymes. Samples with values above 20 mcg/ml were reanalysed after dilution with 0.9% sodium chloride.

APPENDIX-VIII

BIOCHEMICAL PARAMETERS OF CONTROLS, DISEASE CONTROLS AND CANCER PATIENTS

Volunteer No.	Haemoglobin g/dl	Vitamin-A mcg/dl	Lysozyme mcg/dl	Leucocytes Absolute No:(/Cumm)			
				Polymorphs	Eosinophils	Lymphocytes	Monocytes
Normal (males)							
1	13.90	43.20	8.40	4,819	237	2,765	79
2	13.63	35.92	10.50	5,200	160	2,640	0
3	14.51	39.20	9.20	5,900	800	3,200	100
4	13.00	33.12	10.00	4,680	288	2,088	144
5	13.57	30.00	11.20	5,976	249	2,075	0
6	13.34	41.12	8.80	5,412	328	2,296	164
7	14.12	32.12	10.20	5,135	316	2,370	79
(Females)							
1	12.51	32.12	11.40	5,589	324	2,106	81
2	11.78	32.88	10.20	5,016	380	2,128	76
3	12.37	39.20	10.60	5,940	720	2,250	90
4	11.43	43.20	9.40	4,940	456	2,052	152
5	11.79	39.23	8.20	5,040	360	1,728	72
6	11.30	29.99	11.00	4,810	592	1,924	74

Contd..

Non-malignant liver disorders							
(i) Jaundice							
1	8.84	34.02	15.10	5,040	648	1,512	0
2	7.21	27.08	16.30	7,182	1,596	2,622	0
3	9.02	28.11	19.00	4,720	800	2,400	80
4	11.10	32.43	17.20	4,900	420	1,680	0
5	7.93	36.12	15.30	5,184	576	1,440	0
6	8.20	25.12	18.70	5,412	738	1,886	164
(II) Cirrhosis							
1	11.35	28.11	14.00	6,440	552	2,108	100
2	9.14	25.00	13.60	6,461	819	1,820	0
3	7.03	36.11	13.70	6,695	1,648	1,751	206
4	7.21	30.28	13.30	7,300	900	1,700	100
5	8.10	30.66	13.70	7,275	873	1,455	97
Non-malignant GI tract disorders							
(i) Oesophagitis							
1	11.21	30.01	13.30	5,265	810	2,025	0
2	10.60	26.32	12.90	5,092	684	1,748	76
3	10.64	28.00	13.10	4,940	532	2,052	76

Contd..

Malignant Liver disorders							
(i) Liver Cancer							
(a) Primary							
1	6.90	14.21	13.10	5,396	497	1,136	71
2	10.38	12.16	12.90	7,500	1,200	1,200	100
3	9.20	13.21	11.40	8,360	1,210	1,430	0
4	7.30	9.98	16.00	9,360	1,080	1,560	0
5	7.80	11.24	12.20	9,438	968	1,694	0
(b) Secondary							
1	10.23	10.32	15.20	7,625	1,039	2,541	346
2	9.21	13.40	12.20	9,750	1,820	1,300	130
3	8.20	11.26	11.20	7,519	1,236	1,442	103
4	11.00	14.68	12.40	7,920	1,540	1,430	110
Malignant GI tract disorders							
(i) Stomach							
1	9.82	13.12	14.00	8,400	896	1,792	112
2	5.90	14.00	16.00	7,875	630	1,785	210
3	11.10	10.62	13.10	5,856	1,171	1,892	90
4	6.60	9.38	12.90	9,120	1,080	1,560	120

Contd..

(ii) Rectum and Anus							
1	6.80	17.38	12.90	7,700	1,540	1,540	220
2	8.00	16.32	14.00	7,560	1,260	1,680	0
3	7.30	15.46	13.10	8,250	990	1,760	0
4	10.32	12.68	11.40	8,265	0	1,235	0
(iii) Small intestine							
1	10.20	17.63	12.90	6,390	630	1,800	180
2	10.70	13.21	13.10	7,650	918	1,428	204
(iv) Oesophagus							
1	8.72	15.21	12.90	4,250	375	1,563	62
2	10.30	21.34	12.90	11,400	760	2,888	152
3	10.21	18.62	13.10	8,137	760	1,953	0
4	7.60	13.11	16.00	10,333	872	1,245	0
5	8.30	10.21	12.2	8,056	848	1,590	106
6	8.10	16.21	14.00	9,100	1,690	2,210	0
7	10.60	13.12	15.20	8,280	2,040	1,440	240
8	7.20	12.01	14.00	7,725	1,030	1,339	206
9	6.21	20.00	15.20	8,006	1,434	2,390	119
10	6.00	21.22	13.10	6,760	2,288	1,248	104
11	7.40	13.12	14.20	7,956	612	1,428	204

Volunteer No.	Total protein g/dl	Albumin g/dl	Globulin g/dl	Gamma globulin g/dl	IgG mg/dl	IgA mg/dl	IgM mg/dl	Zinc mcg/dl
Normal (Males)								
1	7.471	4.63	2.841	2.500	2,000	255.50	184.3	80.23
2	7.052	4.32	2.732	2.462	2,050	271.30	126.6	74.68
3	5.915	3.99	1.925	1.53				85.21
4	7.032	4.72	2.312	2.15				91.70
5	5.792	3.92	1.872	1.63				82.74
6	6.535	4.41	2.125	2.05				74.89
7	6.99	4.68	2.31	2.10				92.76
(Females)								
1	6.56	4.41	2.15	1.98				76.24
2	5.96	3.98	1.98	1.72				95.27
3	5.58	3.91	1.67	1.48				87.40
4	6.83	4.68	2.15	1.98				91.50
5	6.54	4.23	2.31	2.01				78.92
6	5.981	4.00	1.981	1.73				83.11

Contd.,

Jaundice- Non malignant liver disorders								
(i) 1	6.81	2.92	3.89	3.65	3,100	288.8	185	96.43
2	7.08	3.10	3.98	3.72	2,800	255.5	176.65	72.86
3	6.97	2.96	4.01	3.93				85.49
4	6.92	3.2	3.72	3.56				72.64
5	7.09	3.2	3.89	3.72				69.45
6	6.48	2.5	3.98	3.73				84.67
(ii) Cirrhosis								
1	6.44	2.54	3.90	3.75	3,160	295.8	199.93	71.42
2	6.59	2.87	3.72	3.43	2,700	312.5	216.07	68.78
3	7.53	3.27	4.26	4.08				82.40
4	6.95	3.12	3.83	3.54				72.04
5	7.26	2.64	4.62	4.21				89.24
Non-malignant GI disorders								
(i) Oesophagitis								
1	7.50	3.92	3.58	3.23	2,860	287.5	176.65	69.45
2	7.13	4.12	3.01	2.86				71.05
3	7.06	3.86	3.20	3.01				80.09
(ii) Ulcer								
1	7.96	4.32	3.64	3.21	2,700	295.8	192.05	74.48
2	6.87	3.99	2.86	2.54				65.42
3	7.33	4.41	2.92	2.69				80.52
4	5.45	3.80	1.65	1.43				70.07

Contd..

Liver Cancer (Primary)								
1	5.55	2.32	3.23	3.08	2,200	426	192.05	61.2
2	5.70	2.56	3.14	2.93	1,643	312.5	133.37	48.3
3	5.25	1.97	3.28	2.98				52.3
4	5.37	1.99	3.38	8.12				55.8
5	5.33	2.12	3.12	2.93				45.0
Secondary								
1	6.93	2.62	4.31	4.01	3,450	406	126.58	38.2
2	6.28	2.46	3.82	3.60	3,050	338.4	176.65	58.5
3	6.38	2.42	3.96	3.72				61.3
4	6.82	2.81	4.01	3.90				60.2
Stomach								
1	7.72	3.12	4.60	4.2	3,450	295.8	313.56	48.5
2	6.76	2.16	4.60	4.4	3,650	530	199.93	62.1
3	7.24	2.92	4.32	4.59				62.8
4	7.28	2.87	4.41	4.64				67.2
Rectum								
1	7.28	3.27	4.01	3.92	3,250	352	323.16	43.1
2	6.99	3.01	3.98	3.75				39.2
3	6.88	2.92	3.96	3.72				37.8

Contd..

4)	6.68	2.70	4.01	3.98				52.30
Small intenstine								
1)	7.70	3.14	4.56	4.10	2,800	312.5	440.00	45.21
2)	8.22	3.21	5.01	4.98				55.80
Oesophagus								
1)	7.91	3.27	4.64	4.23	3,450	338.40	241.19	62.10
2)	6.20	2.58	3.62	3.23	2,860	321.1	470.00	62.80
3)	7.33	3.12	4.21	3.79				58.40
4)	6.98	2.72	4.21	3.82				48.30
5)	7.32	2.92	4.40	4.01				37.20
6)	6.91	2.90	4.01	3.98				48.1
7)	7.86	4.00	3.86	3.62				37.3
8)	7.61	2.80	4.81	4.42				41.2
9)	7.55	3.43	4.12	3.92				28.7
10)	6.79	2.71	4.08	3.98				38.4
11)	7.11	3.10	4.01	3.98				70.2