VARIATIONS IN PROTEINS AND ELECTROLYTES DURING STRESS IN HEALTH AND DISEASE IN HUMAN SUBJECTS

Thesis submitted to the
Nagpur University, Nagpur
for the Degree of
DOCTOR OF PHILOSOPHY
in the faculty of Science (Zoology)

BY
Mrs. SHUBHANGI S. PURANIK

RESEARCH GUIDE
DR. (Mrs.) S. A. BHIDE

Department of Zoology
INSTITUTE OF SCIENCE, NAGPUR
MAHARASHTRA (INDIA)

1997

To : **The Registrar,**
Nagpur University, Nagpur.

From : **Dr.(Mrs.) S.A.Bhide**
Reader in Zoology,
Institute of Science,
Nagpur 440 001.

CERTIFICATE

I hereby, certify that this thesis entitled "Variations In Proteins And Electrolytes During Stress In Health And Disease In Human Subjects" in the Faculty of Science, Nagpur University, Nagpur, embodies the results of bonafide research work carried out by Mrs. Shubhangi S. Puranik, under my guidance and supervision. I find the work comprehensive and complete and fit for the purpose of sending it to the examiners.

I, further, certify that such help or source of information that has been availed of in this connection is duly acknowledged and no part of the thesis, whatsoever has been submitted elsewhere for any other degree.

S. A. Bhide

Nagpur
Date : 5·7·97

Dr. (Mrs.) S.A.Bhide
Supervisor

Head of the Department

Director, 5.7.97
Institute of Science
Nagpur.

DECLARATION

I, hereby declare that the work submitted in this thesis was conducted by me during the period of October 1993 to June 1997 under the guidance and supervision of Dr (Mrs.) S.A.Bhide, Reader, Department of Zoology, Institute of Science, Nagpur. It has not been submitted for any other diploma or degree of this university or any other university.

The present work is completely original to the best of my knowledge.

Nagpur

Date : 5-7-97

S. Puranik

Mrs. Shubhangi S. Puranik

Forwarded through the supervisor.

S. A. Bhide

Dr. (Mrs.) S.A.Bhide
Reader in Zoology,
Institute of Science,
Nagpur.

Contents **Page No.**

A. INTRODUCTION

Stress situations may be due to psychic factors, acute physical pain or disease. Stress proteins, or HSp proteins (heat shock proteins) as they are called, have been assigned to specific genes and regulatory elements in humans (Lewin, 1990). It was therefore thought that it would be worth while to study serum protein profiles in an array of individuals under varied stress situations. The subjects chosen for the study of serum protein profiles were

(i) mentally retarded children in whom mental stress was perceived to be negligible (Chapter I).

(ii) students appearing for the first M.B.B.S. examination when they were in great stress, and after the completion of their examination when they were in a relaxed state (Chapter III).

(iii) known cases of depression : subjects who were unable to cope up with acute stress and therefore became depressed (Chapter II).

(iv) women in labour pains before and after normal delivery and also women that delivered by caesarean section, before and after delivery (Chapter II) and finally

(v) patients of acute and chronic renal failure: before and after hemodialysis (Chapter IV)

Since there is scarcely any information on the immune status of mentally retarded children and patients of depression, the total counts of CD_3 positive 'T' lymphocytes and CD_{19} positive B lymphocytes in blood has been studied in mentally retarded children (Chapter I). CD_{16} positive Natural Killer cells have been studied in patients of depression (Chapter 3).

Although several factors are known to be responsible for mental retardation, the levels and role, of calcium and magnesium and the heavy metals, copper, iron, manganese, nickel, lead and zinc has not been studied in mentally retarded children. These have, therefore, been examined in the sera of mentally retarded children (Chapter I).

The extreme outcome of disease and related stress is death. The last Chapter (IV), therefore, deals with lunar periodictiy and sexual, seasonal and circannual variations in deaths due to kidney function disease.

B. CHAPTER 1

Serum Electrolytes, Serum Proteins and Immunocytochemistry of 'T' and 'B' Lymphocytes in Peripheral Blood of Mentally Retarded Children.

The preamble includes general information only on the biological role of the electrolytes Calcium and Magnesium and heavy metals, Cadmium, Chromium, Copper, Iron, Manganese, Nickel Lead and Zinc.

1. PREAMBLE

a) Calcium :

The skeleton constitutes 99% of the body store of calcium. It is primarily in the form of crystals of hydroxyapetite derived mainly from milk. It is important for growth and hence its requirement is more in growing children and pregnant women. It is absorbed at the level of duodenum and upper small intestine and excreted by the routes / ways of urine and feces. Calcium is essential for several biological processes like proper neuromuscular functions, blood coagulation and regulation of secretion of certain hormones like pituitary, parathyroid hormones etc. A fall in plasma calcium below a certain concentration would result in increased release of parathyroid hormone and suppression of release of calcitonin. Conversely, a blood calcium concentration increased above normal would result in augmented secretion of calcitonin and suppression of release of parathyroid hormone (Ahuja, 1989).

b) Magnesium :

Mg is essential for normal growth and development. The vital role of magnesium in cellular metabolism is enzyme activation. The metal seems to activate all enzyme systems which catalyze the transfer of phosphate from ATP to a substrate or from a

phosphorylated compound to ADP. The metal is also required for activation of adenyl cyclase system and for the activity of cyclic AMP dependent kinases. Mg is absorbed from the small intestine and excreted through urine Jaffery et al. (1989).

c) **Heavy Metals :**

Metallothionein is a protein that protects the cell against excess concentration of heavy metals. The protein binds the metal and removes it from the cell. Metallothionein genes are expressed at a basal level, but may be induced to greater levels of expression by heavy metal ions (such as cadmium) or by glucocorticoids (Lewin, 1990).

1) **Cadmium** - Presence of Cd in the environment is of great significance because it has been linked with certain health problems in human beings (Freiberg et al. 1971). It is one of the non-essential elements which may cause serious hazard to human beings if its uptake exceeds the prescribed dietary levels. Inhalation of cadmium oxide fumes causes throat irritation, chest pain, cough and dyspnea (Elkins 1959). Headache, nausea, vomiting, chills, weakness, diarrhea and death from pulmonary edema have also been reported (Spolyar et al. 1944). Ingestion of cadmium causes Itai-Itai and nephritis disease. Cd also causes degeneration of testicular tissue (Scanlon 1975). The most common characteristic of chronic cadmium poisoning is the appearance of a low molecular weight protein in urine (Friberg 1948).

2) **Chromium** - Chromium also is not an essential element for animals. Hexavalent chromium is irritating and corrosive to mucous membrane. It has been confirmed that hexavalent chromium produces lung cancer, ulceration, perforation of nasal septum, respiratory complication and affects skin (Gemell 1972).

3) **Copper** - The total body content of copper in human is 60 to 100 mg. In copper deficiency disorders, there is depressed growth, bone disorders, anemia, neonatal ataxia, impaired reproductive function, gastro-intestinal disorders, heart failure etc. The primary biochemical lesion is drastic reduction in the activity of cytochrome oxidase and amine oxidase. Evidence of copper deficiency syndrome has been obtained in mal-

nourished human infants during the rehabilitation phase on high caloric but low copper diet that may give rise to nephrosis and Wilson's disease. If copper poisoning is severe, hypertension, haemetemesis, melena and coma may supervene (Holtzman et al. 1966 and Oski 1970).

4) Iron - The normal serum ferritin level is 65-170 µg/dl. A greater part of iron in the body is present as hemoglobin. Most of the body iron exists in complex form bound to protein, either as porphyrin or heme or as ferritin and transferrin. Iron deficiency is the commonest cause of nutritional anemia. The deficiency is due to dietary deficiency, malabsorption syndrome, increased demand and pathological blood. As iron deficiency progresses the tissue iron and respiratory enzymes are reduced. Patients complain of lassitude, weakness, fatigue, dyspnea on exertion and palpitation. Finger nails become thin and flattened (Koilonycia). Iron overload is called Hypersiderosis. It may occur as a primary disorder or secondary disorder due to excessive entry of exogenous iron into the body. It causes pigmentation of skin, cirrhosis of liver, pancreatic damage and endocrine disturbances (Sood, 1989).

5) Manganese - Manganese is absorbed by the gastrointestinal tract depending on the solubility of Mn compounds. Mn is retained in soft tissue, mostly in liver and kidney and to a smaller extent in the bone marrow, blood and lung (Cotzias, 1958 and 1962; Schroeder et al., 1966; Underwood, 1977; O'dell and Camphell, 1971). The excess adsorption of Mn causes irritability, difficulty in walking, speech disturbances and abnormal gait. It is also reported that ingestion of water contaminated with Mn results in brain disease, similar to Parkinson's disease (Mena et al. 1967) Vague aches and asthama, headache, muscular weakness, tremors and increased muscle tone are frequently observed. Iron deficiency appears to increase Mn absorption (Mena et al., 1969) The disease caused by excess of Mn is known as ataxia.

6) Nickel - Nickel is poorly absorbed from the normal diet and is excreted in feces. Spectrographic studies have shown that the body does not significantly retain Nickel. In a number of patients with myocardial infarction, values were significantly

higher. The precise biological role of Ni is not known. The metal is a potent activator of several enzymes in vitro (Jaffery 1989).

7) **Lead** - Lead inhibits biosynthesis of heme, particularly in the conversion of delta amino levulinic acids to phosphobilinogen and the formation of heme from iron and protoporphyrin. Thus Lead inactivates the enzymes. Lead poisoning causes loss of appetite, insomnia, bodily discomforts, cramps in leg muscles, constipation, diarrhea and colic abdominal pains (Cantario and Trumper, 1944). It was reported that mothers excessively exposed to lead during pregnancy give birth to stunted and generally abnormal infants (Allaway 1975). The common diseases caused by excessive lead intake are anemia, encephalitis, peripheral neuritis.

8) **Zinc** - Zinc is one of the first trace metals known to be essential for animals. Zn occurs in the body in two different protein combinations.

a) as a metalloenzyme in which Zn is an integral part of an important enzyme system, such as carbonic anhydrase for the regulation of CO2 exchange and

b) as a metal protein complex in which Zn is loosely bound to a protein which acts as its carrier and transport mechanism in the body (Vallee, 1959). Zn deficiency symptoms include loss of appetite, loss of sense of taste and delayed healing of burns, accidental wounds, surgical incisions. It is also reported (Allaway 1975) that Zn deficiency may lead to serious reproductive problems, including infertility of males, failure of conception or implantation of the embryo, difficult birth and deformed offspring. In human diets, meat is an important source of Zn. Dietary zinc levels of 500 ppm do not cause signs of toxicity or other detrimental effects. Excessive Zn intake induces nausea, vomiting cramps, diarrhea and profound lethargy. Since, Zn is absorbed fairly well in the digestive tract, its chronic ingestion has been presumed to be responsible for gastrointestinal disorders (Hamidi, 1962). Inhalation of Zn fumes results in malaria like fever called (Sturgis et al., 1927; Pardera, 1972).

2. INTRODUCTION

Several investigators have studied variations in serum electrolytes and minerals in children. Mishra et al. (1992) studied salivary and serum iron status in children aged 8 months to 10 years with iron deficiency and iron overload. They observed a significant correlation between salivary iron and serum iron. A significant correlation was also noted between protein levels in serum and saliva. Johnson et al.(1992) studied the correlation between decreasing hemoglobin levels and increasing behavioral problems in preschool children of low income group.

Szabe et al. (1989) found that levels of albumin IgG and IgA were low in premature infants. Kugler et al. (1992) noted that children below seven years had lower salivary IgA concentration than children above seven years or adults.

In all these studies and several others, physiological variables were studied in children but there is scarcely any information on the variations in serum electrolytes and serum proteins of mentally retarded children. The present chapter therefore embodies observations on serum electrolytes and serum proteins of mentally retarded children. It also includes studies on total counts of 'T' and 'B' lymphocytes in peripheral blood of 6 mentally retarded and 3 normal children since these have not been investigated so far.

3. MATERIAL AND METHODS

The subjects for this study were 6 mentally retarded children (Table I.1) varying in age from 8 to 14 years from Nandanwan Boys Hostel, Sitabuldi, Nagpur. They were all from low income group families and their mental age was 4 to 6 as reported by the doctors attending on them. 3 normal age matched children served as controls.

5 ml. of premeal venous blood was drawn, collected in a clean sterile glass bulb and allowed to clot. It was centrifuged at 3000 rpm for 10 minutes. The clear serum was separated.

a) Polyacrylamide - bisacrylamide disc-gel electrophoresis (PAGE) - Serum proteins were separated by PAGE from 3 µl samples obtained from 6 mentally retarded children and 3 normal age matched control children on a 1 mm slab gel. Gels were fixed and stained in a solution of 5% Amido Black 10 B and destained in 7% acetic acid. Dark blue bands were apparent after a few hours of destaining but 2-3 days were necessary for the optimum development of clear bands.

b) Immunocytochemistry of 'T' and 'B' lymphocytes in peripheral blood - 1µl premeal blood was drawn from 6 mentally retarded children and 3 normal age matched control children. Blood was distributed into 10-15 dots on a slide to facilitate counting of T and B cells for quick and easy visualization. This method ensures the counting of every cell present in 1µl sample.

For immunocytochemistry, slides were immersed in chilled acetone and stored at O°C. Slides were fixed in chilled acetone for 10 minutes and stored at - 50°C. For staining, slides were brought to room temperature and subjected to endogenous peroxidase blocking for 30 minutes followed by 20 minutes in 20% goat serum. Slides were treated with primary antibody for 1 hour at room temperature/overnight in the

Table I.1 Age, Mental Age of 6 mentally retarded children (1-6) and 3 normal (N1, N2, N3) age matched control children whose serum samples where analysed by PAGE.

S.No.	Age	Mental Age
1	15	6
2	13	8
3	10	8
4	15	6
5	13	8
6	14	8
N1	9	
N2	9	
N3	12	

refrigerator. Secondary Ab used was goat anti mouse conjugated to biotin. Incubation was for 1 hour/overnight. The final incubation was in streptavidin peroxidase followed by 3,3' diaminobenzidine tetrahydrochloride as the chromogen. All immunostained dots of blood films were counter stained with haemotoxylin or Leischmans stain. Blood smears (dots) of age matched normal control subjects were used as positive control while elimination of the primary antibody from dots served as negative control.

Monoclonal antibodies used

Antibody	Specificity	Dilution	Source
SJ25CL	CD_{19}	1:20	Sigma (U.S.A.)
UCHT1	CD_3	1:100	Sigma (U.S.A.)

The number of T and B cells were counted and expressed as the number present in 1 µl blood.

c. **Estimation of Electrolytes** - 1 ml of blood serum sample was taken in a micro Kjeldhal flask and digested by the wet digestion process using nitric and perchloric acid mixture (2:1) at room temperature. When all the organics were digested and emission of white fumes ceased, the digested mass was diluted with double glass distilled water to a known volume.

i) **Calcium and Magnesium** - Calcium and Magnesium was estimated by the EDTA titration procedure and the results were expressed in mg/100ml of serum. To a quantity of digested serum sample ammonium acetate buffer solution was added to maintain the pH of about 10.5 and titrated with standard solution of EDTA using Erichrome black T as an indicator. The calcium was estimated again by EDTA titration technique using ammonium perporate as an indicator and the pH was maintained at about 13.0 so that the magnesium is precipitated and ammonium perporate is left. The latter forms a complex with the calcium ions. The magnesium concentration was obtained by estimating the difference between total EDTA titrant and calcium EDTA titrant. By calculation, calcium and magnesium ion concentration was estimated / 100 ml of sample.

ii) **Heavy Metals (cadmium, chromium, copper, iron, manganese, lead, nickel and zinc)** - The digested sample was also used for the estimation of heavy metals i.e. cadmium, chromium, copper, iron, manganese, lead, nickel, and zinc using atomic absorption spectrophotometer (AAS) under optimum conditions and the results were expressed in mg/100ml. The operating parameters for Hitachi Model Z-8000 Polarzed Zeeman Atomic Absorption Spectrophotometer are given in table I.2.

Atomic Absorption Spectrophotometry techniques are more accurate, precise and reliable than colorimetry and spectrophotometry methods which are time consuming and have limited application.

Atomic absorption is defined as the absorption of radiant energy by atoms. This absorption and its quantitative correlation with the concentration of metal ions originally present in a sample solution serves as the basis of analytical Atomic Absorption Spectroscopy.

d) **Estimation of Hemoglobin** - Haemoglobin content of blood was estimated by the Haldane's method. It is based on the principle of making an acid haematin solution of blood under experimentation in the graduated tube and then comparing it with the sealed comparison tubes containing standard acid haematin. N/10 HCl solution is filled in the graduated tube up to 2 gm mark. The micropipette is filled up by sucking fresh human blood upto the mark of 20 cm. The blood from the micropipette is added to the N/10 HCl solution in the graduated tube. When blood has been expelled, the pipette is rinsed twice or thrice by distilled water. The acid haematin solution is thoroughly stirred with the help of a glass rod and allowed to stand at least for 10 minutes. Subsequently, the acid haematin solution is gradually diluted by adding distilled water in a dropwise manner. With the addition of each drop of distilled water the solution should be stirred and its color matched with that of the standard sealed tubes. This should be continued till the color of the acid haematin solution just fades away as compared to that of the standard comparison tubes. The reading before the color just fades is taken as the correct and final reading.

4. OBSERVATIONS

a. **Serum Proteins by PAGE** - A Comparison of serum protein profiles of 6 mentally retarded children (1 to 6) with a normal child (N), (fig 1.2) demonstrates that the albumin, transferrin, C_1 and gamma globulin bands do not show much variation. The 2 post transferin bands in subject 1 and 3 exhibit a distinct reduction in their quantities.

b. **Immunocytochemistry of T and B lymphocytes in peripheral blood** - It is apparent from Table 1.3 and fig. 1.4 that in any given child the numbers reported for CD_3 positive T lymphocytes and CD_{19} positive B lymphocytes were not at much variance although between individuals there was a large difference. Further, enumeration of T and B lymphocytes demonstrated that the values in mentally retarded children (1 to 6) did not vary much from those of age matched control children (N_1, N_2, N_3). Whereas in some children the absolute T cell count was more than the B cell count, in others the reverse was true and in still others the figures for both were almost the same.

From figure 1.3 a, b, it was observed that from 1 µl blood, all the T - lymphocytes were stained positively (dark brown) with CD_3 surface markers and B lymphocytes with CD_{19} antibody.

c. **Serum electrolytes**

i) Calcium and Magnesium

From table 1.4 and fig. 1.5, it is apparent that the serum concentrations of Calcium and Magnesium is high in mentally retarded children as compared to normal age matched healthy children except subject no. 7 (normal child). An interesting observation is the fact that the latter has a sibling who is mentally retarded. The normal serum Ca concentration is 9-11 mg/100 ml. and Mg concentration is 2-3 mg/100 ml.

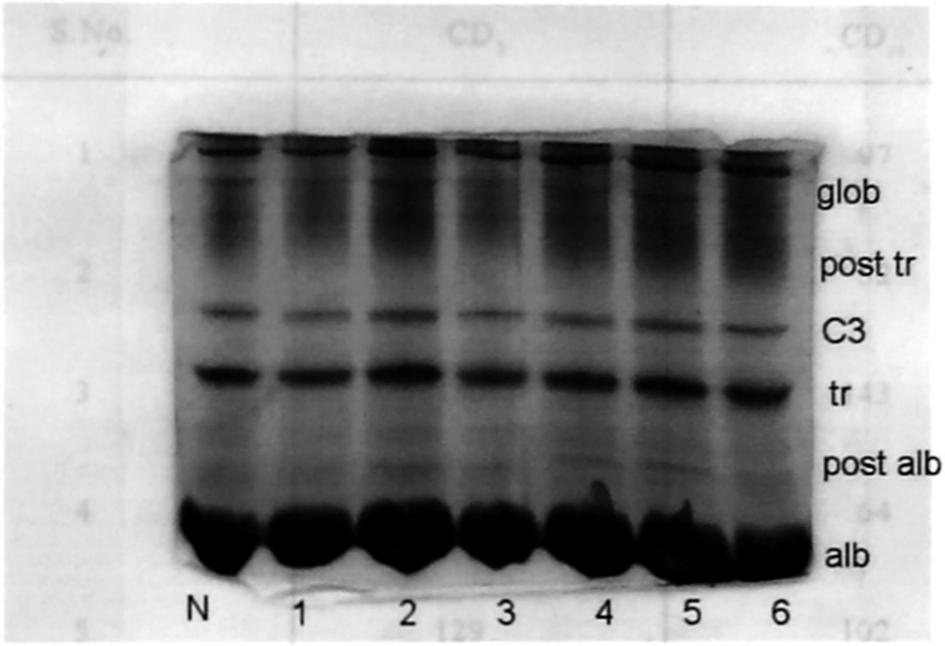


Fig.1.2 PAGE of serum proteins of mentally retarded children.

Table I.3 Absolute count of CD_3 positive T cells and CD_{19} positive B cells in 1 µl blood of 6 mentally retarded children (1-6) and 3 normal (N1, N2, N3) age matched control children.

S.No.	CD_3	CD_{19}
1	109	97
2	56	52
3	180	143
4	52	64
5	129	102
6	178	127
N1	61	68
N2	128	152
N3	72	70

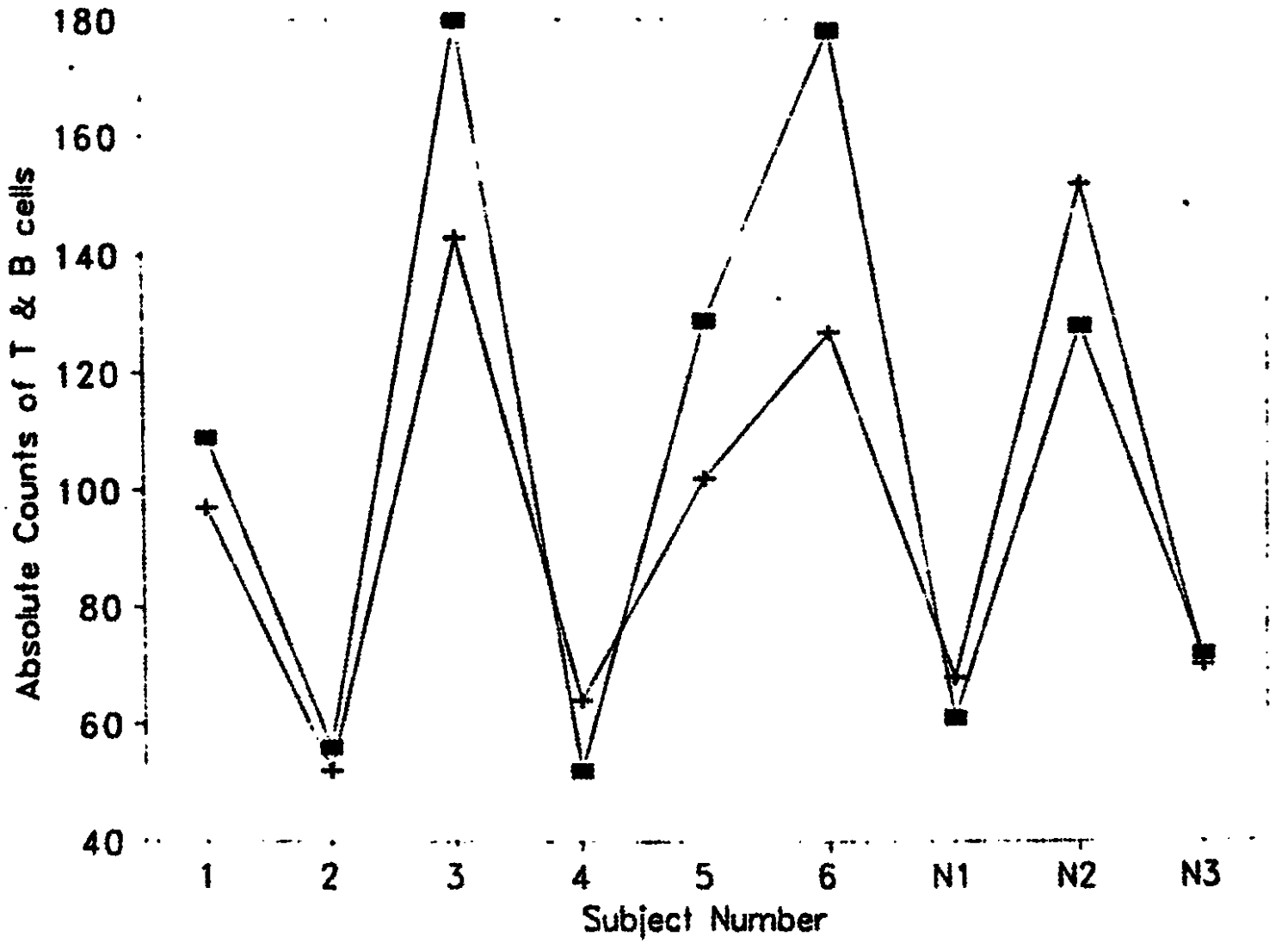
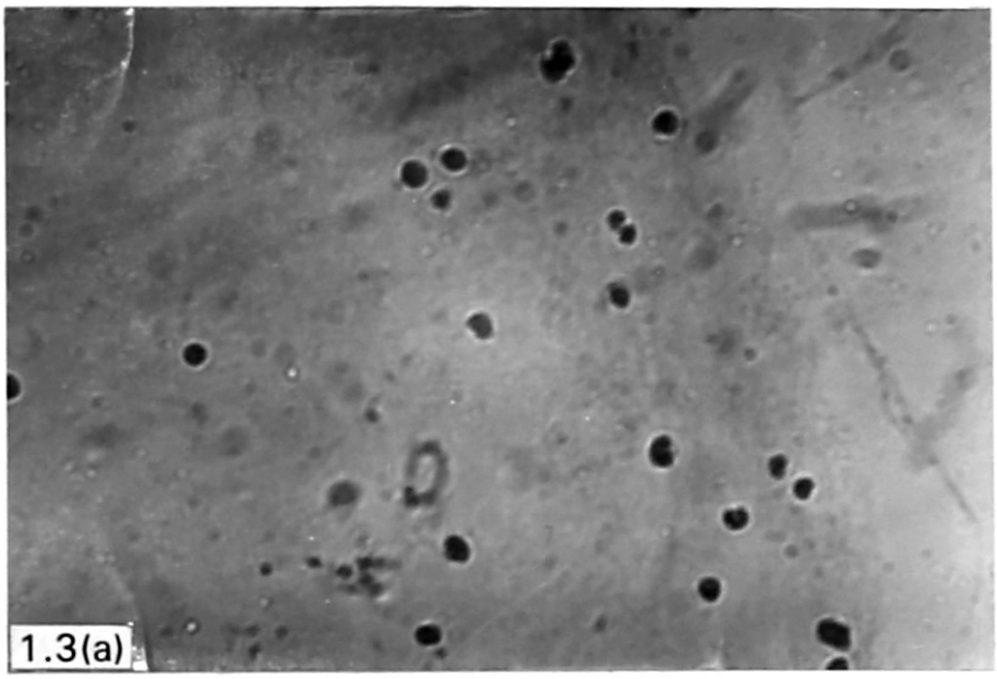
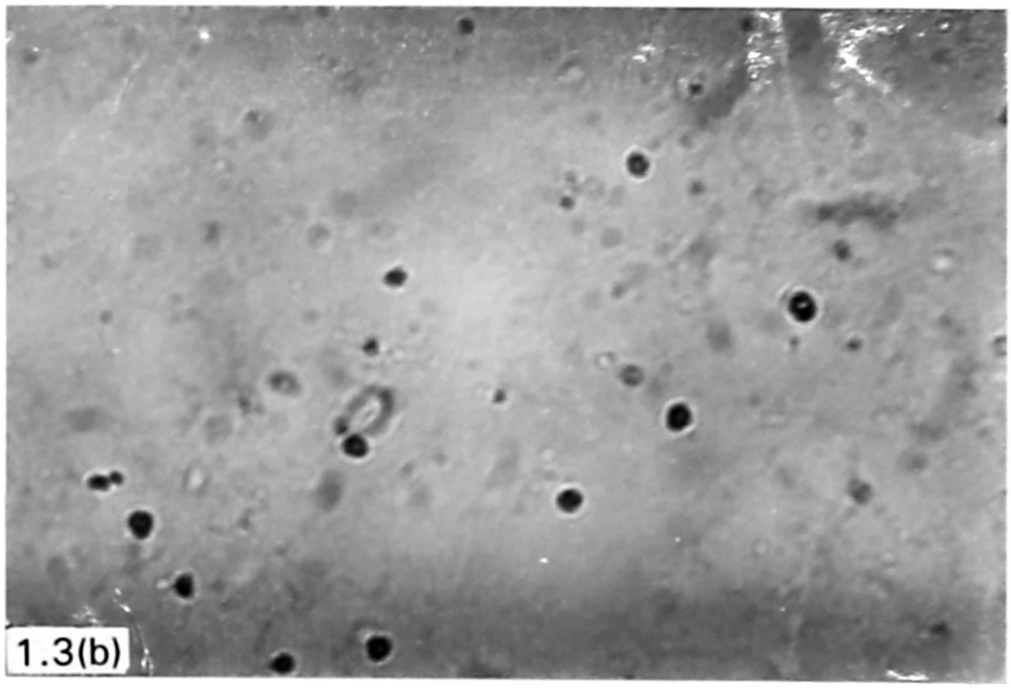


Fig. 1.4 Absolute count, of CD_3 Positive T Cells and CD_{19} Positive B Cells in 1μl blood of 6 mentally retarded (1-6) and 3 Normal (N1, N2, N3) Children.



1.3(a)



1.3(b)

Table I.4 Comparision of concentration of heavy metals and haemoglobin levels in blood serum of 6 mentally retarded (S. No. 1 - 6) and 3 normal children (S. No. 7 - 9). (Their height and weight are also given.)

S No	Calcium mg/ 100	Magnesium mg/ 100	Cadmium ppm	Chromium ppm	Copper mg/ 100	Iron µg/ 100	Manganese µg/ 100	Nickel ppm	Lead µg/ 100	Zinc mg 100	Haemoglobin	Height Feet	Weight Kg
Normal Value	9-11	2-3	0 7	0 009-0 055	0115	65-170	2-3	0 06	< 20	012			
1 MR	14 90	4 83	0 04	9 32	1 32	686	66	0 46	56	0 268	10	5	33
2 MR	18 60	5 60	0 06	2 12	1 30	1446	34	0 42	6	0 554	7 2	4 8	27
3 MR	22 60	6 80	0 06	8 14	1 38	2136	190	Nil	64	0 43	8 4	4 3	23
4 MR	12 80	4 50	0 08	2 82	1 00	1576	98	0 24	138	0 432	10 4	4 9	30
5 MR	13 20	4 80	0 08	2 96	1 40	2152	86	Nil	76	0 94	9 2	411	32
6 MR	15 20	5 00	0 04	9 00	2 16	744	38	0 42	34	0 656	116	411	35
7 N1	14 60	5 00	0 04	1 68	1 24	504	Nil	0 58	Nil	0 098	8 4	4 8	27
8 N2	10 80	3 20	0 04	1 98	1 76	478	Nil	0 88	36	0 064	12 2	4 9	28
9 N3	9 40	2 80	0 06	4 90	0 76	126	Nil	0 34	12	0 168	11 8	5 2	60

* MR - Mentally retarded. N - Normal

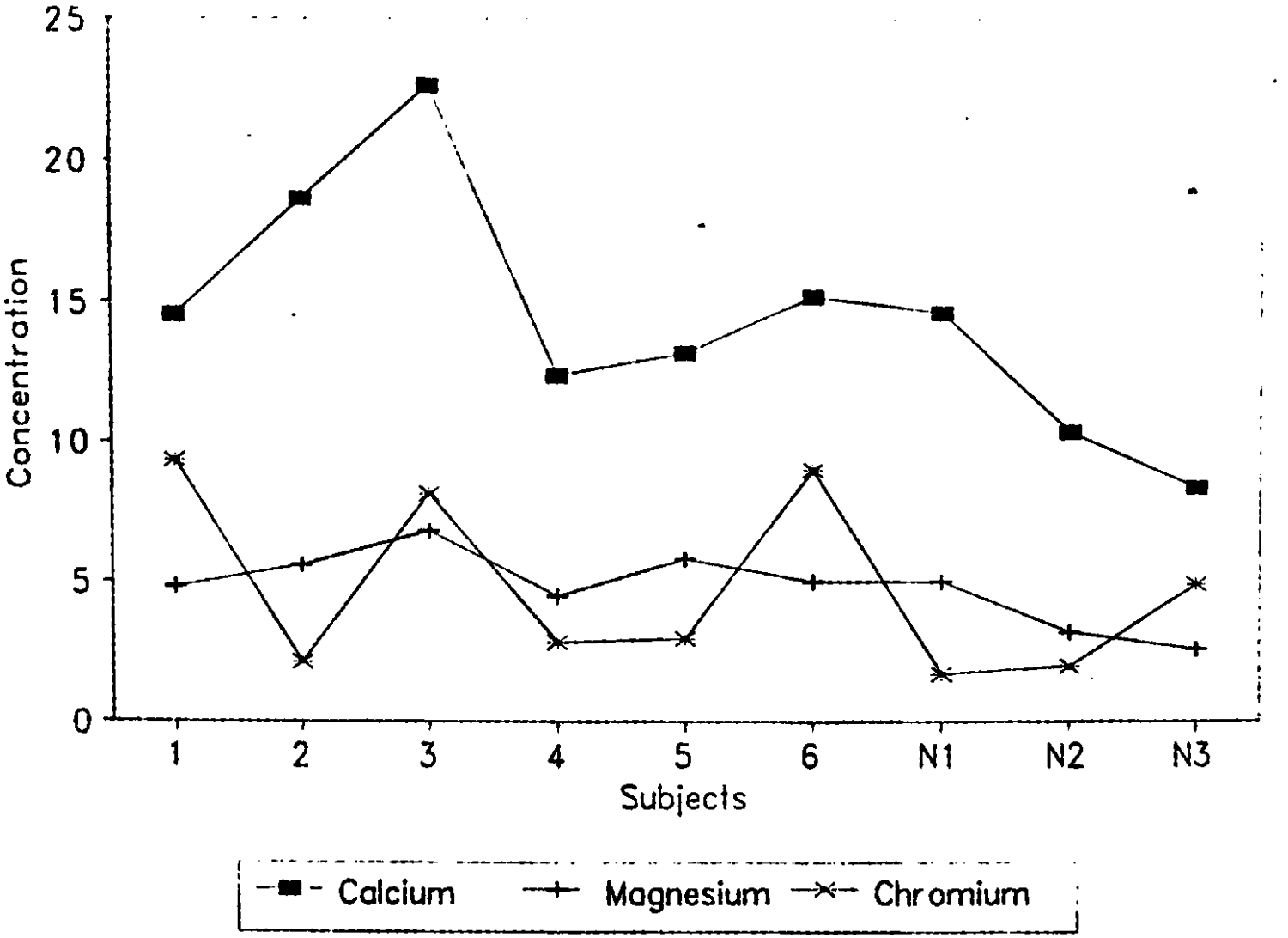


Fig. 1.5 Calcium (mg/dl), Magnesium (mg/dl) and Chromium (ppm) concentration in serum of 6 mentally retarded (1 - 6) and 3 Normal (N1, N2, N3) Children.

ii) Heavy metals - cadmium, chromium, copper, iron, manganese, nickel, lead, zinc.

Cadmium and Chromium - From table I.4 and fig 1.5, fig 1.6 it is observed that the serum Cadmium and Chromium concentration in mentally retarded children does not vary much as compared to the serum levels of these elements in normal healthy children.

Copper - There was not much variation in the serum levels of copper in normal and mentally retarded children in the present study. However, the values observed in the present study vary much from those reported elsewhere (0.115 mg/dl.) for normal adults. (Table I.4, Pg. 1.7)

Iron - An interesting observation is the finding that the serum iron status in most of the mentally retarded children (subject no. 2, 3, 4, 5, table I.4 and fig. 1.7) is extremely high. In the other subjects too (normal subjects 7 and 8) the iron levels were much higher than the values reported for normal children but were lower than those noticed in mentally retarded children.

Manganese - Of all the elements studied Manganese was the only element which was negligible in normal children (subject no. 7, 8, 9, table I.4 and fig. 1.8) 2-3 µg/dl. whereas in mentally retarded children the values were extremely high. This fact was particularly true for subject no. 1 (66 µg/dl), subject no. 3 (190 µg/dl), subject no. 4 (98 µg/dl). The values for subject no. 2, 5 and 6 were 14, 16 and 38 µg respectively.

Nickel - No significant difference was observed in the serum levels of mentally retarded and normal children (Table no. I.4 and fig. 1.6). However, while in two mentally retarded children (subjects 3 and 5) the values are nil, in the other four subjects (1, 2, 4 and 6) the values were higher than those observed for two normal children (subject no. 8 and 9). Interestingly, the serum Nickel levels of subject no. 7 who has a sibling who is mentally retarded is high (0.58). The values observed for Nickel in all

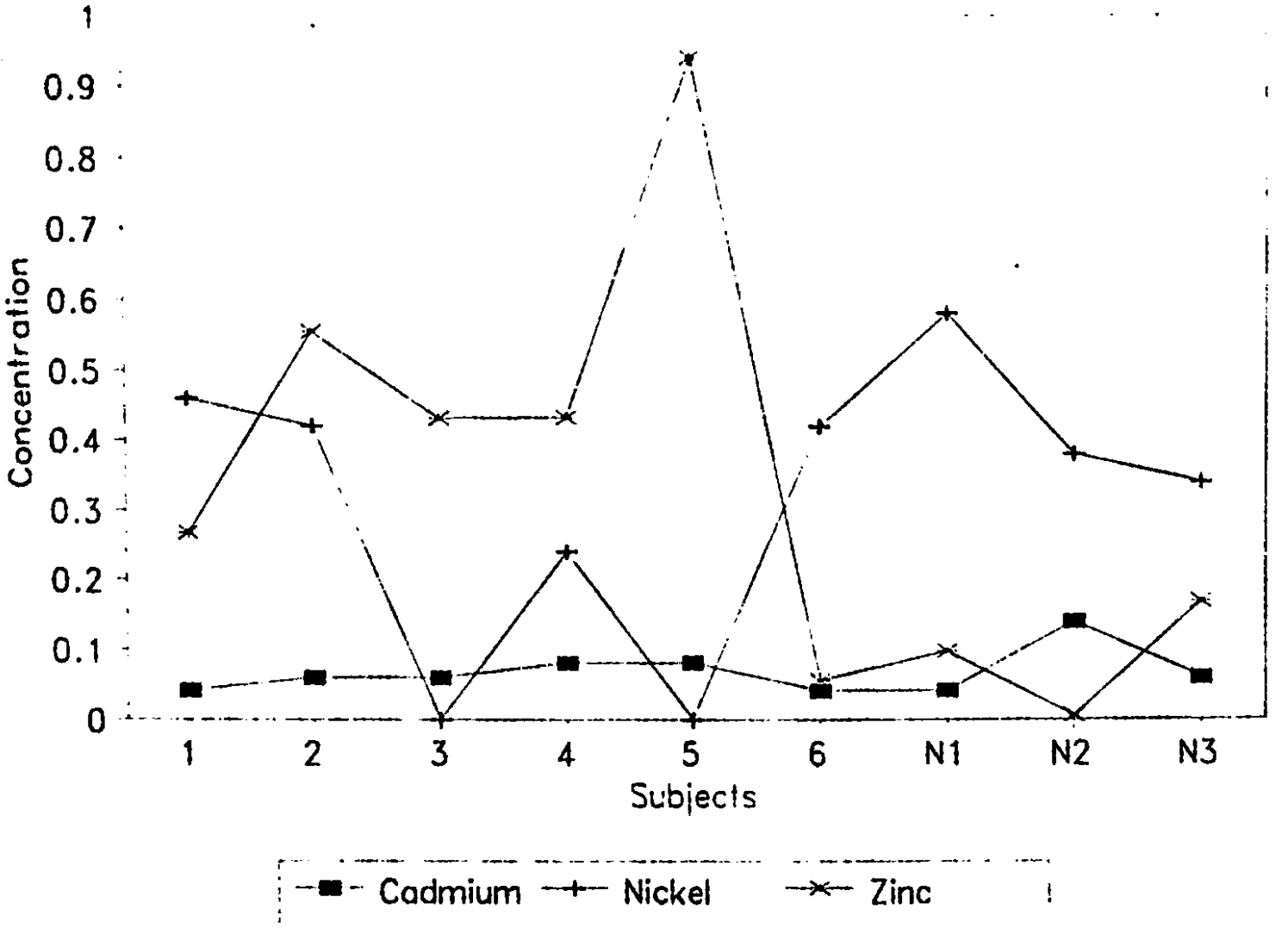


Fig. 1.6 Cadmium (ppm), Nickel (ppm) and Zinc (mg/dl) concentration in serum of 6 mentally retarded (1 - 6) and 3 Normal (N1, N2, N3) Children.

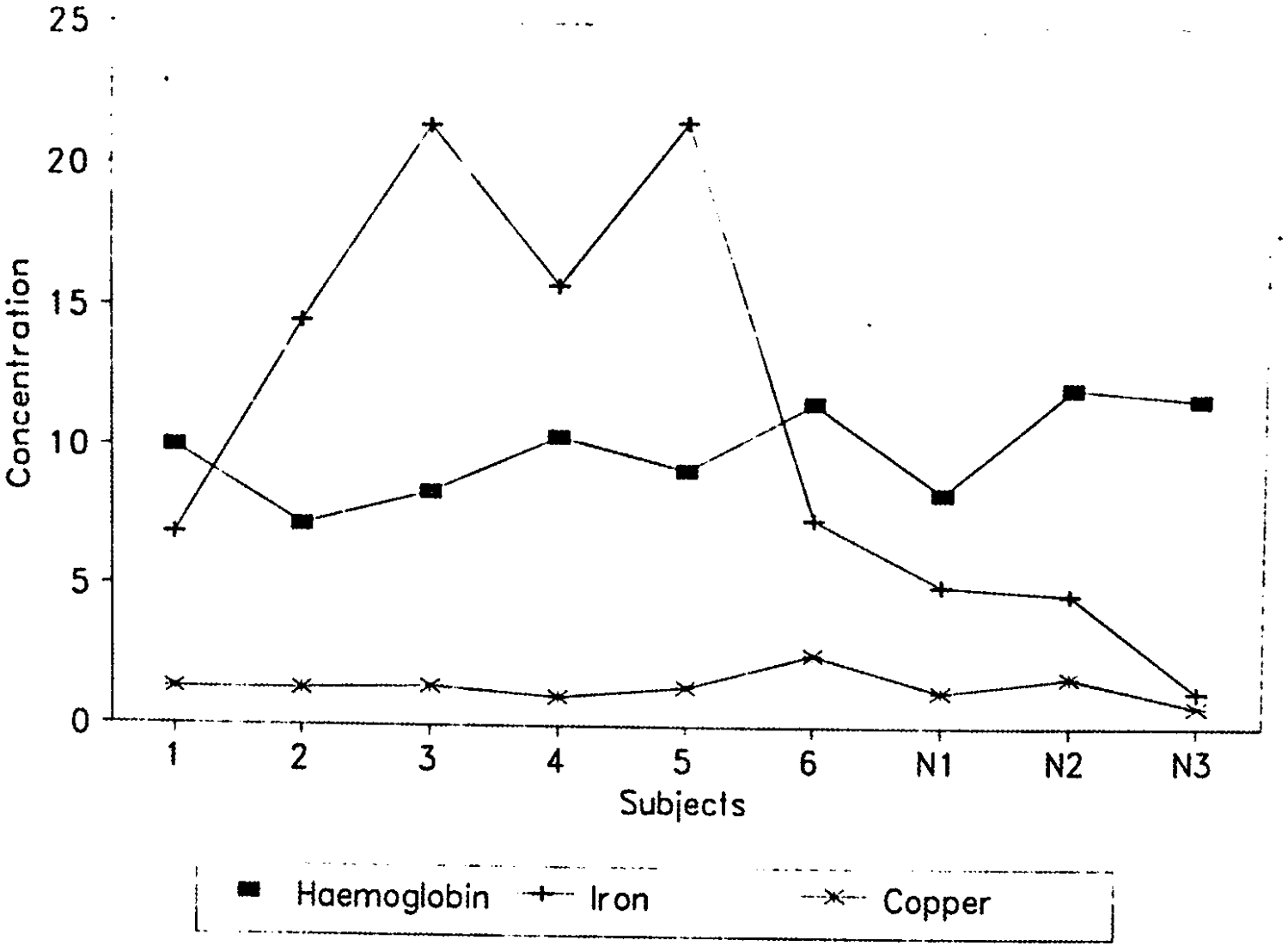


Fig. 1.7 Haemoglobin (mg/dl), Iron (μg/dl) and Copper (mg/dl) concentration in serum of 6 mentally retarded (1 - 6) and 3 Normal (N1, N2, N3) Children.

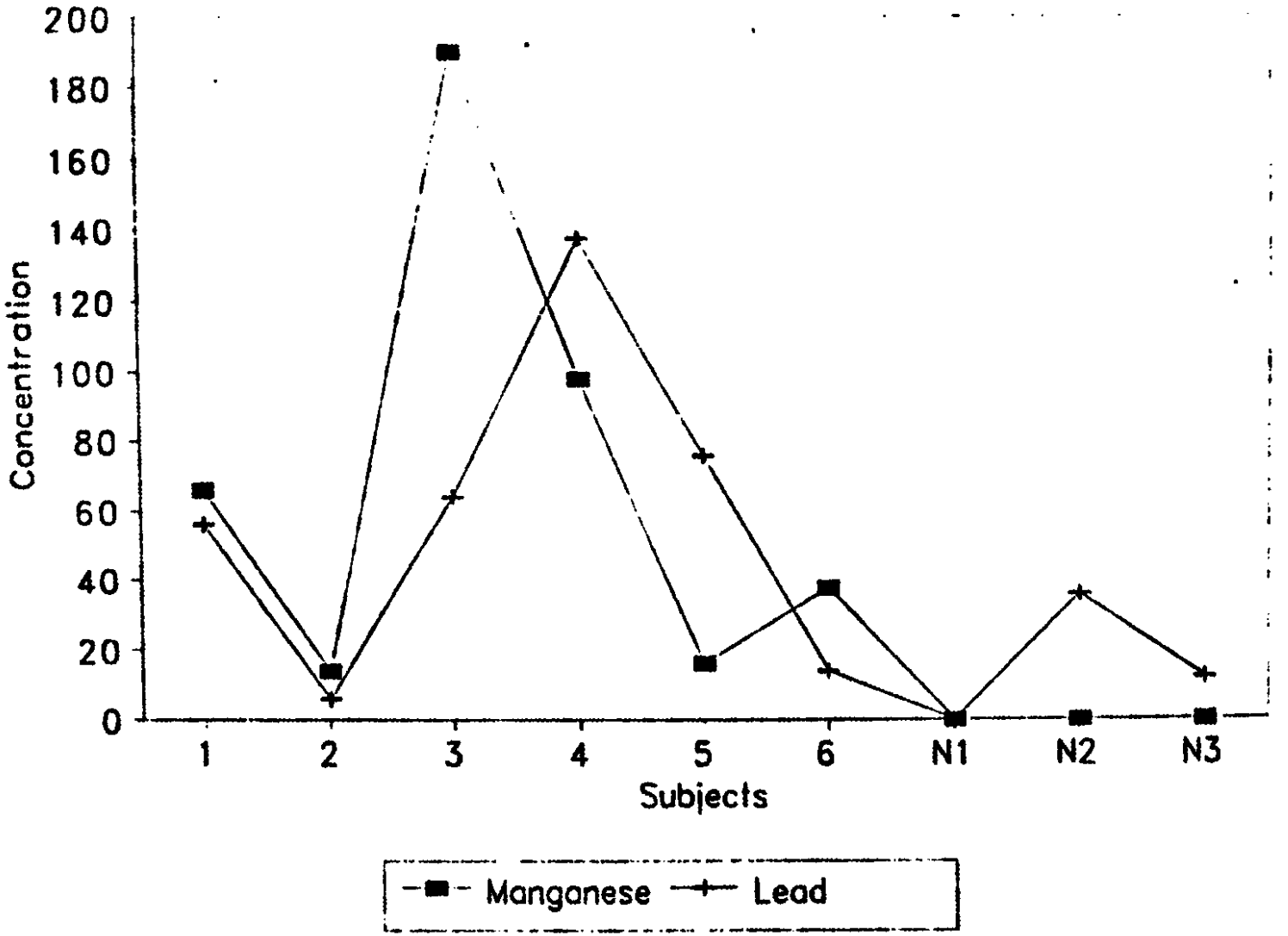


Fig. 1.8 Manganese (μg/dl) and Lead (μg/dl) concentration in serum of 6 mentally retarded (1 - 6) and 3 Normal (N1, N2, N3) Children.

normal subjects and four mentally retarded subjects ~~the subjects~~ were far higher than those reported elsewhere for adults, In two MR subjects the values were nil.

Zinc - Zn levels in the serum of mentally retarded children are high (except subject no. 7) as compared to age matched normal controls (Table no. I.4 and fig. 1.6).

d. **Haemoglobin**- Haemoglobin percentage in most of the children is less than 10 mg/dl and hence they are anemic but in two children the levels are normal. Mentally retarded children have low weight as compared to their age. (Table No. I.4, fig

5. DISCUSSION

Environment affects the psychological development of children. Bad environment, lack of security, love and neglect, all factors contribute for psychological disorders. Many abnormal children have many etiological factors together. The most commonly occurring abnormalities are mental retardation, attention deficit disorder, conduct defect, habitual disorders like nail biting, thumb suckling, enuresis etc. Most of the disorders are originated due to neglect of parents or aggravated due to the same.

Mental retardation is not a disease but handicap either from birth or due to accidents in early childhood. Normally, intelligence of the child is measured by the term Intelligence Quotient. On an average, a normal child has 100 as his I.Q. or may be more. This is calculated by many tests, interviews, and is the comparison of the child's mental age and physical or biological age. When a child has an I.Q. below 70, he is called as intellectually retarded. According to the IQ the children may have mild retardation, moderate retardation, or severe retardation.

The causes of mental retardations, are many, viz. during pregnancy, if the mother faces some problems of basic deficiencies of minerals, or the toxic effects of certain drugs. For example, if Iodine is not available, the child may have cretinism, or hypothyroidism or congenital malformations, during the development of brain of the child. Delayed labour : if there is unusual delay in the delivery, the brain of the child remains devoid of oxygen. This can cause mental retardation. Premature delivery, or intrauterine growth retardation of the child : If the child is underweight, or even after delivery, if the child suffers from infections, like polio, measles, encephalitis and meningitis or if the parents have blood group incompatibility particularly of Rh type. There may be genetical causes, for example, if parents have problem of errors in fat, protein and carbohydrate metabolism. There may be chromosomal aberrations which result in mongolism. Elderly mothers, is also a risk factor, or birth injuries can contribute to this malady

Whatever may be the cause of mental retardation there is no medicine to cure this handicap. However, we can prevent mental retardation. And we can do a great deal for the rehabilitation of these children. The participation of the parents, the special schools, society, occupation therapy, counselors and doctors, is a must to cope with mental retardation.

Genetic Factor :

Willemse et al.(1995) developed a rapid antibody to identify fragile 'X' patients The test was used for screening large groups of people and neonates for fragile 'X' syndrome. They found that the fragile 'X' syndrome is the most common known case of inherited MR.

Godde et al.(1996) observed that an increase in CGG repeat sequences causes an alteration in the functional organization of the chromatin in such a manner that it abolishes the preferential nucleosome assembly. This may cause alteration in the expression of the FMRI gene and the disease phenotype.

Johnson et al.(1995) screened the CGG trinucleotide repeat expansion in the fragile 'X' mental retardation 1 gene in 128 schizophrenic patients. They also reported premutation of the gene in one woman. They concluded that mutation or premutation of the FMR-1 gene was not the only reason (criterion) for the etiology of the vast majority of schizophrenic patients.

Matilainen et al. (1995) studied 151 cases of mental retardation in Finland. They observed that in 28% of the cases mental retardation was due to genetic factors. Of these, trisomy 21, fragile 'X' syndrome and partylglycosaminuria (13%, 4%, 82% respectively) were the 3 most common causes of MR and typical of Finnish in heritance. CHIU et al. (1992) made a chromosomal screening of blood samples of 674 mentally retarded children. They observed 28 Down's syndrome, 4 Klinefelter Syndrome, 1 XYY, one XXX, 11 translocations, 7 inversions, 4 mosaics, 3 duplications, one deletion and one with an extra marker chromosome.

Steftenburg et al. (1995) studied active epilepsy in 98 (35 mild and 62 severe) mentally retarded children aged 6-13 years. They found the prevalence was 2.0/1000 (0.7/1000 for mildly and 1.3/1000 for severely) for retarded children. They reported that 69 children had at least 1 additional neuroimpairment, 42 children had cerebral palsy, 24 had visual impairment and 24 children had autism and 33 children had only a mild or no motor disability. They concluded that active epilepsy in mentally retarded children is often associated with additional neuroimpairments.

Hoekstra et al. (1996) studied the cognitive and psychosocial functioning of patients with congenital nephrogenic diabetes insipidus (NDI). They studied 17 male NDI patients. Total IQ of 14 patients was within (n=13) or above (n=1) the normal range. 1 patient had IQ between -1 and -2 standard deviation and 2 patients had more than 2 S.D. below the normal. No relation was found between test performances and age at diagnosis or hypernatremia. They opined that the current prevalence of MR among patients with NDI was considerably lower than suggested in literature.

Buchner (1996) studied functional principles of molecular chaperons which are sets of conserved protein families that share the remarkable ability to recognize and selectively bind nonactive proteins under physiological and stress conditions. They suggested that the cell developed chaperon families to support protein folding. They found an interesting finding that irreversible unfolding reactions are suppressed by all the major heat shock protein families (HSp 104, HSp 90, HSp 70, HSp 60/GroEL). Chaperons seem to convey thermotolerance and guarantee survival. The molecular mechanism by which they influence protein folding processes is not known.

Devi, and Verrraju (1993) examined the association of ABO and Rh(D) blood groups of 325 MR children with suitable controls. Significant association between MR children for ABO and Rh(O) blood groups was not observed.

It was thought that mental retardation may be reflected in the protein profiles obtained by electrophoretic separation of serum protein. Hence, investigations were carried out in the present studies to examine the serum protein profiles of mentally retarded

children. No difference was observed in their protein profiles as compared to those of normal children.

Behavior

Chaney, et al. (1994) studied the activity and behavioral rhythm disturbances in adults with MR. They observed many wake-sleep and other circadian and behavioral disturbances Few hours of sleep, delays in sleep onset, multiple night time awakenings and abnormal daytime behaviors was noted. They also observed substantial aggressiveness, self-injurious behavior, destructive-tendencies, unacceptable social behavior and frequent stereotype in these subjects.

Piazza et al. 91996) studied sleep patterns in children and young adults with MR and severe behavioral disorders of 51 individuals (age 3 to 21 years). The subjects had less total sleep and less night sleep than peers of the same age and 88% had disturbances of sleep. They found that 'Appropriate' sleep i.e. the amount and regularity of sleep was corrected positively with standard measures of IQ and 'Total sleep' i.e. the overall no. of hours was not correlated with measures of cognitive functioning.

Krauss, (1993) studied similarities and differences between mothers and fathers of 121 children with disabilities. They observed that fathers reported more stress related to their child's temperament and their relationship to their child. Mothers reported more stress from the personal consequences of parenting. They also observed that fathers were more sensitive to the effects of the family environment and mothers were more affected by their personal support networks.

Rodrigue et al. (1993) studied perceived competence and behavioral adjustment of siblings of children with autism. They compared 19 siblings of autistic children with 20 siblings of children with Downs syndrome and 20 siblings of developmentally normal children. They observed more internalizing and externalizing behavior problems in siblings of autistic children than in siblings of developmentally normal children. No significant difference was observed in the 3 groups on measure of perceived self competence or parents report of social competence.

Iida et al. (1994) investigated the mental health of 70 school teachers for MR, 60 care staff in social welfare for MR, 124 nurses and 369 general office workers by general health questionnaire (GHG). They found that :

(1) Mental unhealthiness was 44.6 %, among staff serving the MR which was higher than among nurses and among general office workers.

(2) females tended to be more mentally unhealthy than males among staff serving the MR and among the school teachers for MR.

(3) The 'younger the age and shorter the experience, there was higher incidence of mental unhealthiness.

Drews et al. (1996) studied the relationship between idiopathic mental retardation and maternal smoking during pregnancy. They studied mothers of 221 children with idiopathic MR and the mothers of 400 children attending public school. They found that maternal smoking during pregnancy was associated with the prevalence of idiopathic MR (slightly more than 50%) and children whose mothers smoked at least one pack a day during pregnancy had more than a 75% increase in the occurrence of idiopathic MR. They concluded that maternal smoking may be a preventable cause of mental retardation.

Immunocytochemistry of T and B lymphocytes in peripheral blood :

Infections are known to cause changes in the absolute counts of T and B lymphocyte subsets. Viz AIDS is known to occur in infants and children and the T cells subset abnormalities in children usually resembles those in adults i.e. the absolute number of CD_4 cells is quite low, despite a normal or near-normal total T-cell number.

Epstein-Barr virus infections induce a marked rise in the number of circulating CD_8 cells. In CMV infection there is also a transient decrease in the number of circulating CD_4 cells. In both these infections the increase in the number of CD_8 cells may be

as much as 8 fold. Measles (1) infection and Plasmodium falciparum malaria have been reported to cause a decrease in the absolute number of CD_4. In contrast, some bacterial infections viz. Staphylococcus aureus or Klebsiella pheumonia may result in relative increases in CD_4 cells with CD_4 : CD_8 ratios as high as 20 (Giorgi, 1986).

Plioplys et al. (1994) studied immunoglobulin reactivity in autism and Reff's syndrome. They collected blood samples of 17 patients aged 8.23 years with autism. They noted that B cell number as measured by anti-B, antibodies and B cell function and serum immunoglobulins were normal. Comparison with normal human cerebrellar tissue shows that, there is increased incidence of IgG anti-210 K neurofilament subunit reactivity. IgM anti-210 K reactivity occurred in 53% of the patients and IgG or IgM reactivity against front cortex western blots was not observed. 8 girls with Rett's syndromes were investigated but failed to reveal any abnormalities.

In the present investigations, the purpose of making absolute counts of CD3 +ve T lymphocytes abd CD19 positive B lymphocytes was that it was thought that there could be a relation between the gene for mental retradation and the genes responsible for T and B cell production, and the same if true would be reflected in the absolute counts in MR children. However, marked variation was not observed in the CD_4 and CD_{19} positive T and B lymphocytes of mentally retarded children as compared to those of normal children thereby indicating that the immune status of these subjects is independent of their condition of mental retardation and that the genes are not linked.

If there is a genetic predisposion, the genes responsible or defective genes are transmitted from generation to generation. The prevalence of mental retardation is 1% in the population. The possibility becomes twice if one of the parents is mentally retarded. If both parents are mentally retarded then possibility of mental retardation is 15%. In the first degree relatives the possibility is always high as compared to the normal population. Since there is similarity at the genetic level, there is an impact over metabolic processes also. If a boy is mentally retarded, his brother or sister also has a more or less similar metabolic setup though he/she is not mentally retarded but he/she

also has the potential for mental retardation if the environment becomes adverse (Ahuja, 1989).

A similar situation obtain in subject 7. The child is a 13 year old boy who has a sibling (10 year old brother) who is mentally retarded, but subject 7 is not mentally retarded. Hence he should be protected from ill psychological environment.

Stressful events in life, affect the life-style of people. Children especially are more susceptible to these events. Dixon and Abrens (1992) observed self reported depression in 84 children between the age of 9 and 12 before and after exposure to stressful events.

In children the prevalence of stress depression relationship is difficult to calculate. Stressful events enter the mind of the child at subconscious level and reflect by the episodes of enuresis, frightening in sleep, nail biting, thumb sucking and various sleep disorders.

Holt and Bang (1986) investigated enuresis among 3,131 children aged 7.2 - 13.1 years. They observed that 121 children had nocturnal enuresis, 16 had both day and night wetting and 12 children had day wetting.

Kulpmann and Gerlach (1996) measured total magnesium concentration by flame atomic absorption spectrophotometer and it was noticed that total and ionized Mg levels were closely related in marked hypermagnesemia (71.2 mmol/L) but the correlation was poor in samples with slightly elevated total concentration or in hypomagnesemia (<0.65 mmol/L). The relationship was dependent on protein concentration. Studies on paraproteinemic sera clearly demonstrated that albumin concentration is most important for the size of the protein bound fraction.

Reunanen et al. 1996 studied the association of serum Ca, Mg, Cu and Zn concentration with cardiovascular mortality. An interesting fact was observed by these workers. High serum Cu and low serum Zn concentration were significantly associated

with an increased mortality from all cardiovascular diseases and from coronary heart disease in particular. No significant association was found with serum Ca and Mg and mortality risk.

Nikolova and Kavaldzhieva (1994) studied the serum and urinary levels of calcium, iron and copper by using AAS but phosphorus content was estimated by the colorimetric methods in 42 workers. They observed that serum phosphorus concentration was significantly reduced, iron and copper slightly decreased and calcium level remained unchanged. They also reported increased P and Ca excretion in urine.

In the present study, serum levels of calcium and magnesium, heavy metals (Cu, Cr, Ni, Pb, Mn, Zn, Fe) and haemoglobin were estimated. Correlation of serum Ca, Mg, heavy metals and haemoglobin in mentally retarded children was also studied. An interesting finding was the observation that in most of the cases, the levels of heavy metals are significantly high in mentally retarded children. Further, Mn was the only heavy metal which was negligible in normal children but in high concentration in mentally retarded children. In most mentally retarded subjects the Fe concentration is extremely high. The levels of Ca and Mg too are high compared to the levels of normal healthy children. A striking fact was noticed during the present studies. The serum levels of all the electrolytes in subject 7 (normal child) were in between the values of those noted for mentally retarded children and normal children. Subject No. 7 has a sibling brother who is mentally retarded. Although subject no. 7 is not a mentally retarded child the serum levels of Ca, Mg, Fe and Zn were high as compared to the other two normal children.

60% of mentally retarded children studied here were anemic i.e. with < 10% Hb concentration. Subject No. 7 has low Hb level (8.6%).

Mishra et al. (1992) studied the salivary iron status in children (8 months to 10 years) with iron deficiency and iron overload. They observed that salivary iron was significantly higher in iron deficient and iron overload conditions compared to controls. They also observed a significant correlationship between salivary iron and serum iron

and found that the mean salivary serum iron ratio was the same in control and iron overload cases and it was twice as high in iron deficient anemic children. Significant correlation between salivary protein level with serum albumin and serum protein was also observed.

Diurnal salivary fluoride concentration in children living in areas with low or high fluoride concentration in drinking water was determined by Olivery et al. (1990). They reported that the mean salivary fluoride concentration was 0.32 ± 0.013 mmol/L in low fluoride (LF) area and 0.87 ± 0.047 mmol/L in high fluoride (HF) area. They could not find a rhythm for diurnal variation in mean or individual fluoride concentration but found fluctuations in the HF areas.

Kugler et al. (1992) studied the secretion of salivary immunoglobin A in relation to age, saliva flow, mood states, secretion of albumin cortisol and catecholamines in saliva. They found relationship between age and salivary IgA concentration. Children below 7 years have lower salivary IgA than children above 7 years or adults. They observed a significant inverse relationship between saliva flow and salivary IgA concentration and states that gender, mood states, salivary albumin, salivary catecholamines and salivary cortisol were nor associated with salivary IgA.

Srivathana et al. (1996) described a new method for measuring capillary blood lead levels in children by filter paper method. They measured Pb level of 100 children aged 9 months to 6 years. The sensitivity, specificity and positive predictivity of the capillary filter paper method relative to the reference method were studied. They found that the capillary filter paper assay had a sensitivity of 90% and specificity of 90% for differentiating blood lead levels of 0.48 µmol/L or more. Blood lead levels of 0.72 µmol/L or more and 0.96 µmol/L or more were identified. They concluded that capillary filter paper method provides convenient, sensitive, accurate and inexpensive method to examine children for elevated blood lead levels.

Shimonura and Kunishisa (1991) studied the influence of crying on plasma renin activity (PRA) and plasma aldosterone concentration (PAC) in infants and toddlers.

They compared the changes of PRA and PAC, one minute after ouset of crying and three and 5 minutes after continuous crying. They observed that both PRA and PAC increased with elapsing time. After a short episode of crying there was marked increase in PRA but a mild increase was observed in PAC.

Several investigative procedures are carried out in order to diagnose the behavioral problems. Haemoglobin percentage of the child has been reported to be correlated with behavioral problems. Johnson et al.(1992) studied the correlation between haemoglobin and behavioral problems in preschool children of low income group. They found that among 2 to 3 and 4 to 5 year old children, there was a significant correlation between decreasing haemologbin values and increasing behavioral problems. They also found significant inverse correlation between haemoglobin and depression and sleep problems in 2 to 3 year old girls and between haemoglobin and aggregation and hyperactivity in 4 to 5 year old girls.

Mishra et al. (1992) studied the salivary iron status in children with iron deficiency and iron overload. They selected children aged from 8 months to 10 years and observed that salivary iron was significantly higher in iron deficient and iron overload conditions compared to controls. They observed a significant correlation between salivary iron and found that the mean salivary serum iron ratio was same in control and iron overload cases and it was twice as high in iron deficient anemic children. They also found a significant correlation between salivary protein level with serum albumin and serum proteins.

Endocrine causes are also suspected for psychological disorders. There are evidences of circannual and circadian rhythms in relation to the endocrinological status of the psychologically abnormal child.

Nicolau et al.(1989) studied the chronobiology of endocrine system. They studied 194 children, 43 young adult and 149 elderly subjects. They found that all subjects follow a diurnal activity pattern. They collected blood and urine samples at 4 hour intervals and noted circadian rhythms of 22 endocrine parameters in plasma and 5 in

urine. They studied circadian rhythms in children and the elderly subjects and found circannual rhythms in many parameters. They also found that elderly subjects showed remarkable maintenance of circadian time structure and circannual periods.

6. SUMMARY

(a) Serum protein profiles of 6 mentally retarded children did not show much variation from those of age matched normal control children.

(b) Immunocytochemistry of CD3 positive T lymphocytes and CD19 B lymphocytes too demonstrated that there was not much difference in their absolute count as compared to those normal control subjects.

(c) No correlation was observed either between the immune status of the subjects or their mental age with their serum levels of electrolytes.

(d) Most mentally children had low Hb concentration (min. 7.2 max 11.6 gm.) but values for free iron were extremely high as also the levels of Ca and Mg.

(e) An interesting finding was the fact that Mn levels were negiligible in normal subjects but in mentally retarded children the levels of Mn were very high.

7. REFERENCES

AHUJA, M.M.S. 1989. Section V, Chapter 45. Calcium and Phosphorus Metabolism, Vitamin D, Calcitonin, Parathyroid Hormone. In : Text Book of Biochemistry and Human Biology Eds. Talwar, G.P., Shrivastava, L.M. and Moudgil, K.D., Prentice - Hall of India, 1989 i-xxvii, PP. 1248.

ALLAWAY W.H. 1975. The Effects on soil and fertilizer as Human and Animal Nutrition Agriculture. Information Bulletin No.378, 31.

BUCHNER, J., 1996. Supervising the fold : Functional principles of molecular chaperons. FASEB.J. 10 (1) : 10-19.

CANTARIO, A. and M. TRUMPER. (1944). Lead poisoning. Williams and Wilkins Co., Baltimore, 143.

CHANEY, R.H., C.E. OLMSTEAD and C.A. GIVENS. 1994. Activity and behavioural rhythm disturbances in adults with mental retardation. DEVELOPEMENTAL BRAIN DYSFUNCTION. 7(1) : 17-25.

CHIU, P.CHIN, Y.S. YUH, C.K.PERNG, S.Y.LI, W.W.SHENG and K.D.WOO. 1992. Chromosomal screening of mental retardation school children in Teipei. J. FORMOSAN, MED. ASSOC. 91(12) : 1162-1165.

COTZIAS, G.C.(1958). Physical Review. 38, 503.

COTZIAS, G.C. (1962). Manganese in Material Metabolism. Vol. 2B. Academic Press, New York, 404-433.

DEVI, K.S. and P.VEERRAJU. 1993. Blood groups and mental retardation. BIONATURE. 13(2):317-319.

DIXON, J.F. and ARHENS. 1992. Stress and attributional style as predictors of self reported depression in children. GOGNIT. THER.RES., 16(6) : 623 - 634.

DREWS, C.D., C.C. MURPHY, M.Y. ALLSOPP and P.DECOUFLE. 1996. The relationship between idiopathic mental retardation and maternal smoking during pregnancy. PAEDIATRICS. 97(4) : 547-553.

ELKINS, H.B. 1959. The Chemistry of Industrial Toxicology. John Wiley and Sons, New York.

FRIBERG, L. 1948. J. Industr. Hyg.. 30-33.

FRIBERG , L., M.PESCATOR and G.NORDBERG. 1971 . Cadmium in the environment. Chemical Rubber Co. Press, Cleveland, Ohio.P-166.

GEMELL, R.P. 1972. Nature (London). 240, 567.

GIORGI, J.V., 1986. Chapter 33. Lymphocyte Subset Measurements : Signigicance in Clinical Medicine : pp 242-243. In Manual of Clinical Laboratroy Immunology. Eds. Rose, N.R. Friedman, H., Fahey, J.L. American Society for Microbiology.

GODDE, J.S., S.U.KASS, M.C.HIRST and A.R. WOLFFE. 1996. Nucleosome assembly on methylated CGG triplet repeats in the fragile X mental retardation gene I promoter. Journal of Biological Chemistry. 271(40): 24325-24328.

HAMDI, E.A. 1962. Bril. J.Ind.Med., 126.

HOEKSTRA, J.A., A.F.V. LIEBERG, L.A.H. MONNENS, G.H.H. DIRKMAAT AND V.V.A.M. KNOERS. 1996. Cognitive and Psychosocial functioning of patients with congenital nephrogenic diabetes insipidus. Journal of Medical Genetics. 61(1): 81-88.

HOLT, J. AND O.BANG. 1986. Prevalence of enuresis among school children 7.2 - 13.1 years old in Bodo (Norway). TIDSSKR. NOR. LAEGEFOREN. 106(8): 650 - 651.

HOLTZMAN, N.A., D.A.ELLIOT and R.H.NELTER. 1966. New Eng. J.Med.. 275-347.

IIDA, J., H.ORIBE, T.HIROSE AND G.IKAWA. 1994. Investigations of mental health of school teachers for the mental retardation and staff members in social welfare facilities for the mentally retarded. JAP. J. OF PSYCHIATRY AND NEUROLOGY. 48(1) : 65-70.

JEFFERY, N.F., R.P.CENTRE and B.L. JAILKHANI. 1989. Section V, Chapter 47. Metabolism of other minerals. In: Text book of Biochemistry and Human Biology Eds. Talwar, G.P., Shrivastava, L.M. and Moudgil, K.D., Prentice - Hall of India, 1989. i-xxvii, PP 1248.

JOHNSON, E., E.BJORKK, J.WAHLSTROM, P.GUSTAVSSON and G.SEDVALL. 1995. Screening for CGG trinucleotide repeat expansion in the fragile X mental retardation 1 gene in schizophrenic patients. PSYCHIATRIC GENETICS. 5(4) : 157-160.

JHONSON, S.R., M.A. WINKLEBY, W.T. BOYCE, R. MCLAUGHIN, R.BROADWIN and L. GOLDMAN. 1992. The association between haemoglobin and behaviour problems in a sample of low-income Hispanic preschool children. J. DEV. BEHAV. PEDIATR. 13(3) : 209 - 214.

KRAUSS, M.W.. 1993. Child related and parenting stress : Similarities and differences between mothers and fathers of children with disabilities. AM. J. MEN. RETARD. 97(4): 393-404.

KUGLER, J., M.HESS and D.HAAKE. 1992. Secretion of salivary immunoglobulin A in relation to age, saliva flow, mood status, secretion of albumin, cortisol and catecholamines in saliva. J. CLIN. IMMUNOL. 12(1) : 45 - 49.

KULPMANN, W R. and M.GERLACH. 1996. SCAND> J. CLIN. LAB. MED. 56(suppl. 224):251-258.

LEWIN, B., 1990. PART 7, CHAPTER 29 Building the Transcription Complex. pp 559 IN : Genes IV by Benjamin Lewin. OXFORD UNIVERSITY PRESS. pp 857.

MANLAINEN, R., E. AIRAKSINEN, T.MONONEN, K.LAUNIALA and R KAARIAINEN. 1995. A population based study on the causes of mild and severe mental retardation. ACTA.PAEDIATRICA. 84(3) : 261-266.

MENA, J., O.MARIN, S.FUNZALIDA and G.C. COTIZAS. 1967. NEUROLOGY. 17,128.

MENA, I., K.HARRICUCHI and G.C. COTZIAS. 1969. NEUROLOGY. 19, 1000.

MISHRA, P.O.,K.N. AGRAWAL and R.M.D. AGRAWAL 1992. Salivary iron status in children with iron deficiency and iron overload. J.TROP.PEDIATER. 38(2) : 64 - 67.

NICOLAU, G.Y. and E.HAUS, 1989. Chronobiology of endochrine system. REV. ROUM MED. ENDOCRINOL. 27 (3) : 153 - 184.

NIKOLOVA, P. and B.KAVALDZHIEVA. 1994. Mineral metabolism of workers in an aluminium ware plant. TRACE ELEMENTS AND ELECTROLYTES 11(4): 174-177.

O'DELL, B.L. and B.J. CAMPHELL 1971. Trace Elements : Metabolism and Metabolic functions . In "Comprehensive Biochemistry". Vol. 21. Elsevier Publishing Co., Amsterdam 179-266.

OLANIYA, M.S. 1990. Thesis on "Studies on heavy metals content in city refuse compost and sewage sludges and their impact on environment. Osmania University Hyderabad

OLIVEBY, A., S.TWETMAN and J.KESTRAND. 1990. Diurnal fluoride concentration in whole saliva in children living in a high and a low fluoride area. CARIES. RES. 24(1) : 44-47.

PAZDEROVA, J. 1972. EXPERTA. MED. (AMST.) 25, 141.

PIAZZA, C.C., W.W. FISHER and S.W.KAHNG. 1996. Sleep patterns in children and young adults with mental retardation and severe behaviour disorders. DEVELOPEMENTAL MEDICINE AND CHILD NEUROLOGY. 38(4) : 335-344.

PLIOPLYS, A.V., A.GREAVES,K.KAZEMI and E.SILVERMAN. 1994. Immunoglobulin reactivity in autism and Rett's Syndrome. DEVELOPMENTAL BRAIN DYSFUNCTION 7(1): 12-16.

REUNANEN, A., P.KNEKT, J.MARNIEMI, J.MAKI, J.MATTELA. and A.AROMAA. 1996. Serum calcium, Magnesium, copper and zinc and risk of cardiovascular death. EUROPEAN JOURNAL OF CLINICAL NUTRITION. 50(7) : 431-437.

RODRIGUE, J.R., G.R.GEFFKEN and S.B. MORGAN. 1993. Perceived competence and behavioural adjustment of siblings of children with autism. J. OF AUTISM AND DEVELOPMENTAL DISORDERS. 23(4) : 665-674.

SCANTON, J.W. 1975. Rev. Envivon. Hlth, 2, 39.

SCHOREDER, H.A., J.J. BALASSA and I.H. TIPOTON. 1966. J. CHRON. Dis., 19 , 545.

SHIMOMURA, K. 1991. Plasma renin activity and plasma aldosterone concentration. Influence of crying. FUKUOKA. ACTA. MED. 82(12). 671 - 679.

SOOD, S.K.. 1989. Section V. Chapter 46, Iron Metabolism: In: Text book of Biochemistry and Human Biology Eds. Talwar, G.P., Shrivastava, L.M. and Moudgil, K.D., Prentice- Hall of India, 1989 i-xxvii, PP 1248.

SPOLYAR; L.W., J.F.KAPLER and H.G.PORTER 1944. J. Indian Hyg. Toxicol., 26, 232.

SRIVUTHANA, K., H.Y.YEE, K.BHAMBHANI, R.MELTON, P.M.SIMPSON and R.E.KAUFFMAN. 1996. A new filter paper method to measure capillary blood lead level in children. ARCHIVES OF PEDIATRICS AND ADOLESCENT MEDICINE. 150(5): 498-502.

STEFTENBURG, U., G.HAGBERG, G.VIGGEDAL AND M.KYLLERMAN. 1995. Active epilepsy in mentally retarded children. ACTA. PAEDIATRICA. 84(10): 1147-1152.

STURGIS, C.C., P.DRINKER and R.M. THOMSON. 1927. J. Ind. Hyg., 32, 1758.

SZABO, S., M.VARGANCSIK - MOSONI, V.NAGY and A.SZABO. 1989. Study of serum and secretory immunmoglobulins in premature infants. REV. PEDIATR. OBSTET. GINECOL. SER. PEDIATR. 38(1): 27 - 32.

UNDERWOOD, E.J. 1977. "Trace elements in Human and Animal Nutrition", 4th edn. Academic Press, New York.

VALLEE, B.L. 1959. Physical Rev., 39, 443.

WILLEMSEN, ROB, S.MOHKAMSING, B.DE VRIES, D.DEVYS, A.V.D.

OUWELAND, J.L.MANDEL, N.GALJAARD and B.OOSTRA. 1995. Rapid antibody test for fragile X-syndrome, Lancet. 345 (8958): 1147-1148.

C. CHAPTER 2.

Variation in Serum Protein Profiles of Women in Acute Stress Situations : Before and After Normal and Caesarean Delivery.

I. INTRODUCTION

A review of the literature reveals that not only anatomical but also physiological and biochemical changes during pregnancy and delivery have been studied extensively.

Electrolytes like Mg, Ca, Fe etc. change in concentration according to trimesters (Goel et al. 1991, Martinez et al. 1986). Changes in proteins have also been reported to occur from trimester to trimester. A new pregnancy associated protein (PAP) has been reported in pregnancy sera (Majima et al. 1991). The relation between serum iron, hemoglobin and ferritin has also been studied during pregnancy (Kaufer et al. 1990). Circadian rhythm of cortisol has also been noticed in pregnant women.

Stress which is a psychological reaction to fear, wonder, shock etc. has an impact over pregnancy and vice-versa. Anxiety and depression are variants of stress. The levels of all hormones have been reported to increase by more than 50% during labor (Shalev et al. 1985). Diurnal, circadian and circannual variations in cortisol have been studied (Perez - Lopez et al. 1984, Cousins et al. 1983, Allolio et al. 1990, Junkermann et al 1982). Tei et al. (1989) studied plasma levels of fibrinopeptide A, fibrinopeptide B, kininogen and kinin during pregnancy, labor, in full term normal delivery and caesarean section delivery.

In all these studies, the physiological and biochemical variables were studied on a number of subjects in different stages of pregnancy and postpartum delivery.

However, variations in different fractions of serum proteins have not been studied in pregnant women before and after delivery. The aim of this study was to analyze the serum protein profiles of women —

(i) who were in acute stress i.e. in labor pains

(ii) who subsequently gave birth by normal delivery

(iii) women prior to delivery by Caesarean section and were not in labor pains and

(iv) after Caesarean delivery.

2. MATERIAL AND METHODS

3ml blood samples were collected from women, a few hours or (just) before delivery i.e. during labor pains and 1 or 2 days after normal delivery. Blood samples from women prior to and after Caesarean delivery were also collected. All samples were collected from Matru Sewa Sangh, Hospital, Nagpur, Table II.1 and were placed for 1 hour for settling. Serum proteins were analyzed by PAGE as described in Chapter I.

Table II.1 Age, Gravid para of 9 women with Normal delivery and 6 women who delivered by Caesarean section.

Sub. No.	Age	Gravid Para	Type of Delivery
1	28	2nd	Normal
2	25	1st	Normal
3	22	1st	Normal
4	21	1st	Normal
5	22	2nd	Normal
6	22	1st	Normal
7	30	2nd	Normal
8	26	2nd	Normal
9	23	1st	Normal
10(1)	25	2nd	Caesarean
11(2)	25	1st	Caesarean
12(3)	28	2nd	Caesarean
13(4)	30	2nd	Caesarean
14(5)	24	2nd	Caesarean
15(6)	21	1st	Caesarean

3. OBSERVATIONS

a. Protein profiles of serum samples before and after normal delivery

Comparison of the protein profiles in serum samples before and after delivery revealed that prealbumin, albumin and transferrin bands were present in both situations but differences are seen in post transferrin bands. The hemoglobin band is visible in haemolysed blood samples.

Fig 2.1, fig. 2.2 and fig. 2.3 it is observed that the post transferrin bands were missing or very faint in serum samples taken before delivery i.e. when women were in acute labor pains whereas these bands are clearly seen in serum samples taken from the same subjects after normal delivery i.e. when they are in a relaxed state. Of the 9 women studied, subjects 2,3,4,6 and 9 were primigravid and subjects 1,5,7 and 8 were parous.

b. Protein profiles of serum samples before and after Caesarean delivery.

Fig. 2.4 illustrates the PAGE of 6 µl serum proteins of 4 women (1, 2, 3, 4) before (B) and after (A) Caesarean delivery, while fig. 2.5 illustrates the PAGE of 12 µl serum proteins of subject 1, and 6 µl serum proteins of two women (5, 6) before and after Caesarean delivery. In subjects 1 and 5 most of the globulin fractions of serum proteins show reduction in quantities and is not visible hence 12 µl serum sample was taken (fig. 2.5) and the serum proteins were separated by electrophoresis. It was observed that inspite of doubling the protein load the 7S globulin fraction was not visible.

In subject 2 there appears to be a general depletion of all protein fractions after Caesarean delivery as compared to the protein profile before delivery. In subjects 3 and 4 there was no noteworthy difference between the protein profiles of samples before and after Caesarean delivery.

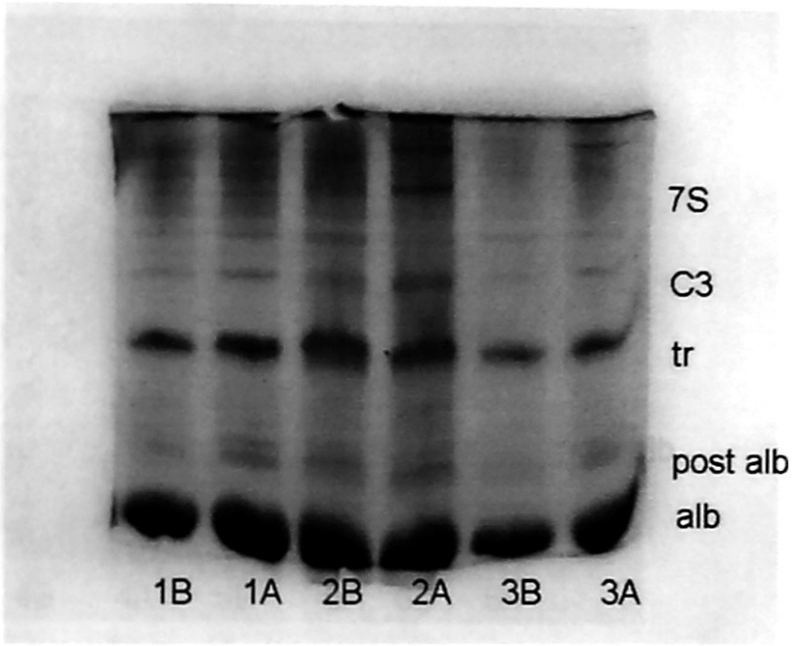


Fig.2.1 PAGE of serum (6 µL) proteins of women (1,2,3) before (B) and after (A) normal delivery.

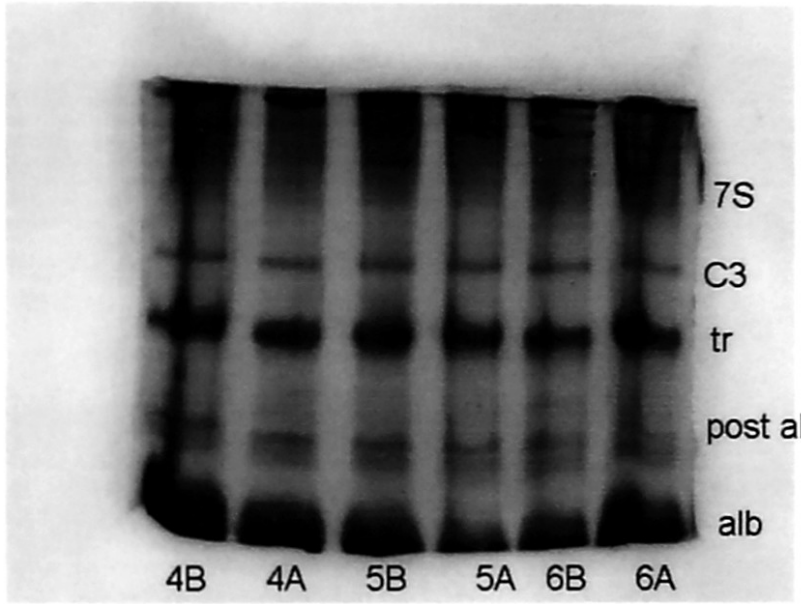
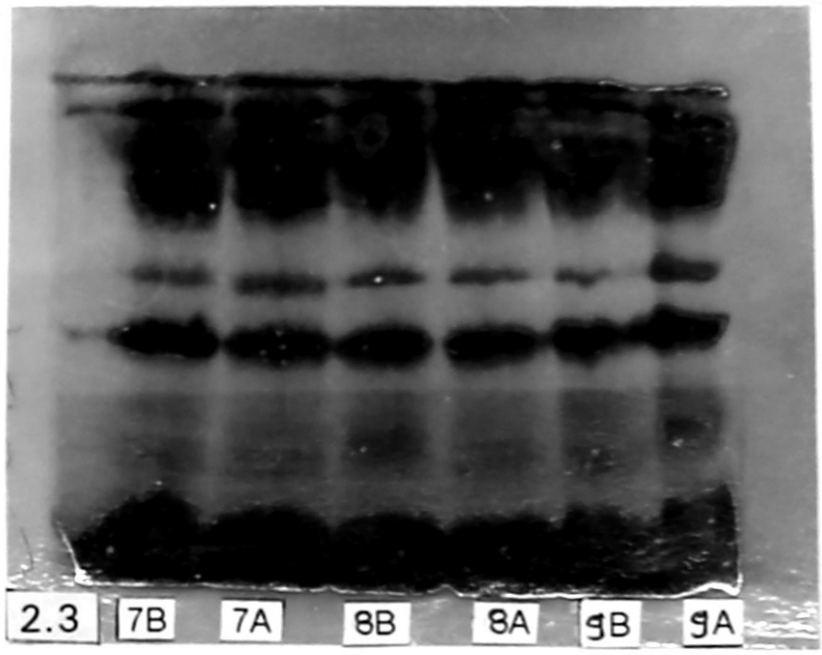


Fig.2.2 PAGE of serum (6 μL) proteins of women (4,5,6) : before (B) and after (A) normal delivery.


2.3
7B
7A
8B
8A
9B
9A

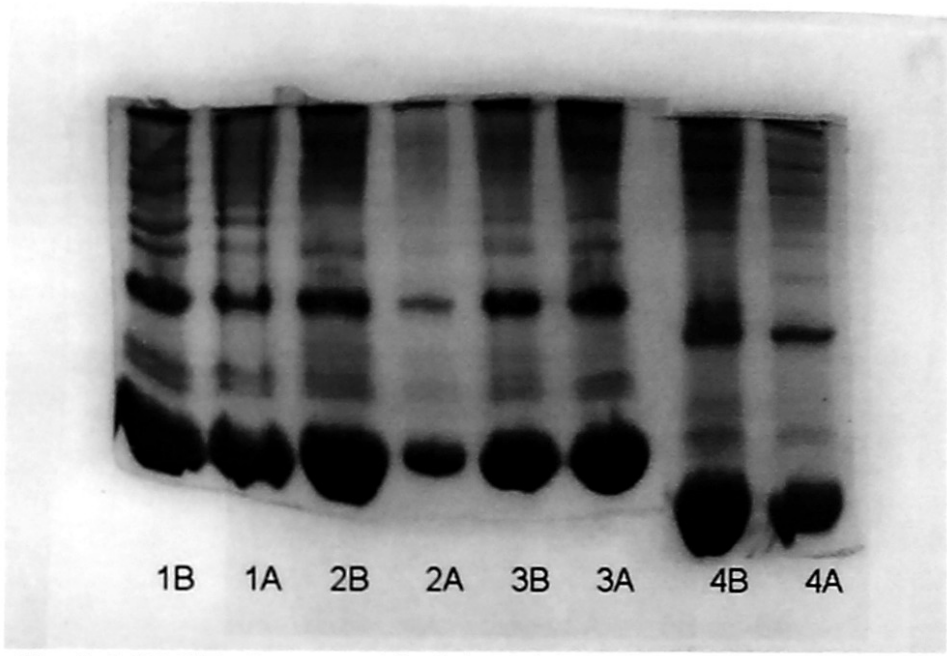


Fig.2.4 PAGE of serum (6 µL) proteins of women (1,2,3,4) before (B) and after (A) caesarian delivery.

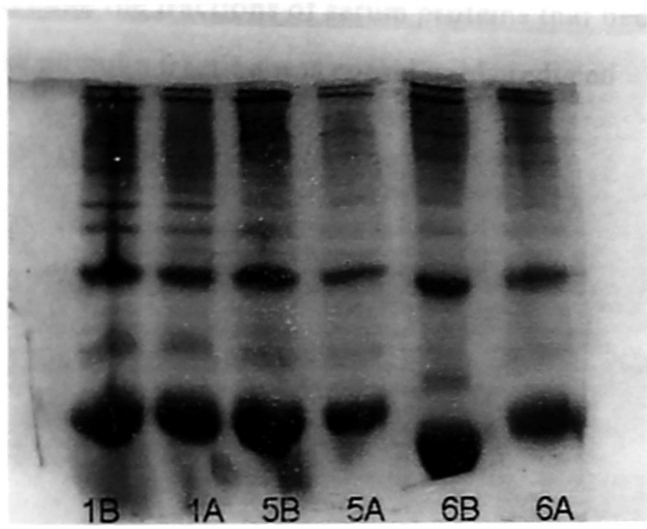


Fig. 2.5 PAGE of serum (6 µL) proteins of women (5, 6) before (B) and after (A) caesarian delivery. (12 uL serum sample was run in well 1A.

In subjects 5 and 6 similar findings as those in subject 2 were noticed i.e. the quantities of serum proteins of most fractions after delivery were considerably less as compared to those observed before delivery.

The protein profiles must be viewed in the light of the fact that serum samples were drawn just prior to delivery by Caesarean section and one day after delivery.

When the protein load was reduced to half (fig. 2.6 i.e. 3 µl serum from peripheral blood drawn from women before delivery) and run on acrylamide gels, in order to determine the fractions of serum proteins that became depleted, it was observed that the globulin fraction was considerably reduced.

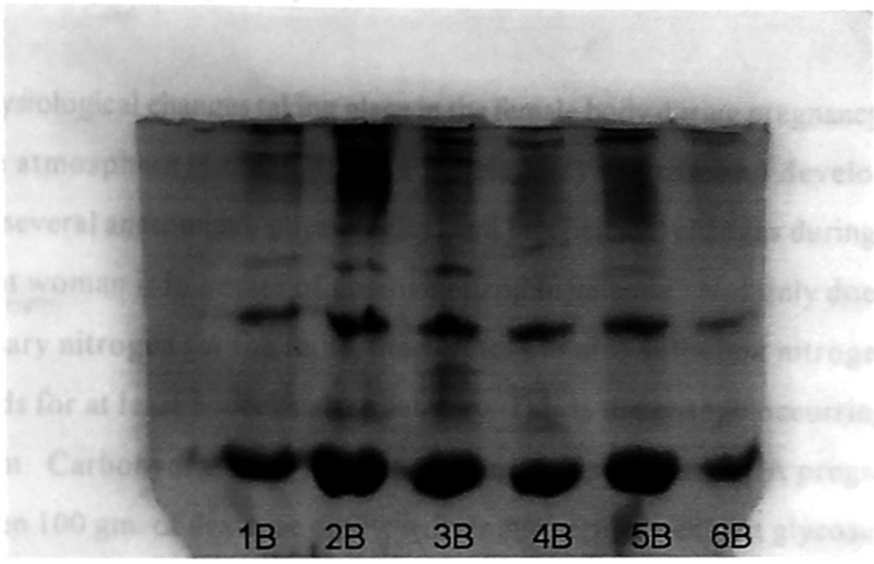


Fig.2.6 PAGE of serum (3 µL) proteins of women (1,2,3,4,5,6) : before (B) normal delivery.

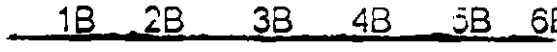


Fig.2.6 PAGE of serum (3 µL) proteins of women (1,2,3,4,5,6) : before (B) normal delivery.

4. DISCUSSION

Literature is replete with information on varied aspects of pregnancy.

a. Electrolytes in pregnancy

Physiological changes taking place in the female body during pregnancy are drastic. Favorable atmosphere is created in the female body to grow and develop the fetus. There are several anatomical, physiological and biochemical changes during pregnancy. A pregnant woman is in a state of positive nitrogen balance. Not only does she retain the necessary nitrogen for the fetus, uterus etc. but also sufficient nitrogen for lactational needs for at least 6 weeks after delivery. This is the change occurring in protein metabolism. Carbohydrate and fat metabolism are also affected. A pregnant woman who is given 100 gm. of dextrose orally, will almost certainly exhibit glycosuria whereas a non pregnant woman will not. The glycosuria of pregnancy has usually been described to a lowered renal threshold. So also the total serum lipids increase by about 50%. Blood cholesterol may rise from a normal level of 150 mg to 350 mg % or more. Tracer studies have apparently disposed off the belief that there is a retention of sodium in normal pregnancy. About 500 milliequivalent sodium are retained during the 2nd and 3rd trimesters and this would be consistent with the expansion of the volume of extracellular fluids (Johnstone and Kellar, 1982).

Other electrolytes like Mg, Ca, Fe etc. are also changed in concentration in blood in pregnant women and the concentration also changes according to trimesters. Goel et al. (1991) studied serum magnesium level in pregnancy. They observed that serum Mg level was significantly lower in pregnant women going into spontaneous labor than present in non pregnant healthy women, normal pregnant women and women in spontaneous labor at term. They concluded that hypomagnesemia was an important factor for spontaneous labor. Lower concentration of calcium ion was observed in second and third trimesters of pregnancy and at delivery than in the control group. Preterm labor is the labor which starts before completion of 36 weeks of gestation. Serum

magnesium levels continuously declined until 33 weeks of pregnancy but after that it was not depressed with the onset of labor at term (Kurzel and Richard, 1991). It has been reported that patients in preterm labor have a significantly depressed serum magnesium level.

Greater concentration of Ca ion was observed in the samples from umbilical vein and artery and from newborns 2-5 minutes after birth. In new borns 40-50 hours after birth, lower concentration than control group was observed (Martinez et al. 1986) Raman et al. (1991) measured plasma calcium and magnesium levels in 341 pregnant and 15 non-pregnant women in different trimesters of pregnancy. 57 women suffered from pregnancy induced hypertension (PIH) and 37 were eclampties. They concluded that low plasma calcium and magnesium levels are indicative of low bone mass.

During pregnancy the muscle mass, soft tissue mass, glands and fats are increased hence the bone mass relatively becomes lowered and that leads to lower levels of calcium and magnesium in the blood (Johnstone and Kellar, 1982).

Relationship of maternal and newborn serum ferritin concentration was studied by Ilyes et al. (1985). They observed that the concentration of ferritin was higher in cord serum than in respective maternal and infant specimens. Mothers with low serum ferritin concentration delivered newborns of lower iron stores than in newborns of mothers who had normal ferritin levels.

Cord blood is the only source of energy to the fetus, and fetal circulation depends on cord blood. Hence the latter is the only site for storage of iron. Therefore, ferritin concentration in the cord blood is higher. Carpeny et al. (1992) determined plasma and cell ferritin in normal fetuses at different gestational ages and evaluated fatal iron status. They observed increased plasma ferritin and red cell ferritin during pregnancy and showed correlation to hemoglobin and number of red blood cells. Experimental evidence showed that placenta stores and transports iron.

The relation between serum iron, hemoglobin and ferritin has been studied dur-

ing pregnancy (Kaufer et al. 1990). They took serum samples 4 weeks before their last menstrual period and at 8, 12, 16, 20, 24, 28, 32 and 36 weeks of pregnancy, during labor and one month after delivery. They observed that Hb decreased significantly during the first week of pregnancy while ferritin increased followed by a gradual fall as pregnancy progressed in women with high ferritin. They also reported that Hb increased during the first week of pregnancy while ferritin remained unchanged throughout the pregnancy in women with a low ferritin. Iron deficiency developed during pregnancy. They therefore suggested an increased utilization of iron stores.

Ferritin is stored iron. The iron requirements increases due to pregnancy and therefore Hb concentration decreases after conception. However, there is no immediate decrease in the levels of ferritin since some time is required for the conversion of ferritin into iron.

Comparisons of minerals, electrolytes and proteins in maternal blood, cord blood and placental blood have been studied. Serum concentration of Ca, Mg and Zn of maternal blood were significantly lower than that of cord blood whereas serum concentration of Cu in maternal blood was significantly higher than in cord blood (Zhou et al. 1991). It has been observed that the concentration of serum Ca and Mg between maternal and cord blood were highly correlated but there was no significant correlation between concentration of Cu and Zn in maternal and cord blood. Serum zinc levels were low during first and second trimester of pregnancy but higher in the third trimester and significantly high during labor (Sharma et al. 1991).

b. Proteins during pregnancy

Laminin is secreted by placenta, hence it is a good parameter of placental function. It has been observed that Laminin increases during pregnancy, about doubles at the time of delivery and the levels drop after delivery. Serum Laminin levels decreased in placental insufficiency whereas increased concentration was observed in sera of preeclamptic women (Bielmayer et al. 1986). At the time of delivery there is hyper functioning of placenta which is aggravated if hypertension is associated. A high mo-

lecular weight pregnancy associated protein which is an angiotensinogen represents the major component of total angiotensinogen. It has been noted that these proteins are produced in placental basement membrane or in the maternal uterine tissue (Tewksbury et al. 1986). Placental protein 14 (pp-14) has been observed to be associated with late pregnancy when measured in 496 women. A slight reduction in the levels was observed in pregnancies complicated by hypertension (Howel et al. 1986).

During pregnancy, the changes in proteins occur from trimester to trimester. A new pregnancy associated protein (PAP) has been reported in pregnancy sera. It was observed that the levels began to rise and reach a plateau in the second trimester, whereas in nonpregnant control sera PAP was below the detection limit (Majima et al. 1991). Bersinger et al. (1986) observed a consistent rise in pregnancy specific proteins sp1 and human chorionic gonadotrophins (HCG) and sharp increase of pregnancy associated protein (PAP-p). In all cases between 6 and 10 weeks but no consistent behavior was observed with advancing pregnancy in $_2$ PAG (pregnancy associated gonadotrophins.

Haller et al. (1988) measured a fetoprotein and pregnancy specific ß1 glycoprotein (SP1) in sera between the tenth and sixteenth gestational ranges week and determined 10 and 9 percentiles as reference levels.

Gerhard et al. (1991) determined the early pregnancy factor (EPF) activity during 6th to 9 th week of gestation and observed that the EPF activity was highest during 6th week of gestation. It has been reported that when pregnancies ended in abortions, EPF was never detected or disappeared 1-2 weeks before abortion. The risk of abortion was 7.6 (first determination) or 20.0 (second determination) in absence of EPF activity. The risk was lowered when pregnancy parameters were depressed. High risk pregnancies and pregnancies following treatment for sterility had high prognostic value.

Pregnancy induced hypertension, does not usually occur until after mid-pregnancy and in the majority of cases not until after the 13th week. The blood pressure (B.P) is more than 140/90 mm of Hg and there is edema and albuminuria. The basic

cause of this hypertension is not known. But Sumioki et al. (1989) determined the mean 24 hour values of atrial natriuretic peptide in mild and severe pregancy induced hypertensions (PIH), pregnancy aggravated hypertension, chronic hypertension and normal pregnancy. Significantly, higher values were observed in mild and severe PIH and pregnancy aggravated hypertension. They found a clear circadian rhythm with acrophase in the middle of the night in mild and severe PIH and no confirmed circadian rhythm in the other hypertensive disorders. It has been concluded that the elevated values of plasma atrial natriuretic peptide during pregnancy was related to generalized vasoconstriction and that the diurnal rhythm was a specific characteristic of PIH. Cimellaro et al. (1985) observed that in severe and mild eclamptic patients, plasma proteins and pregnancy specific glycoprotein (_2 PAG) levels were below the lower limit and a significant increase of plasma ceruloplasmin level was noted. It has been suggested that the trophoblast was the only source of pregnancy associated plasma protein A in vitro (Barnea et al. 1980). It has been observed that an 85 KD immunoregulatory glycoprotein called uromodulin, from pregnancy urine binds to and regulates the activity of interleukin I. It has been suggested that there are a no. of phenomenon during pregnancy that are regulated on account of molecules that specifically modulate interleukin 1 and 2 (Muchmore 1986). During normal delivery, significant increase of fibrinopeptide A (FPA), fibrinopeptide B ß 15-42 (FPB ß 15-42), and kinin was observed. However, during Caesarean section an increase of FPA in uterine venous blood was noticed. A significant decrease of high molecular weight kininogen (HMW-Kg) during normal labor and of prekallikrein and kallikrein inhibitor after caesarean section have also been reported (Tei et al. 1989). The hypercoagulate state during pregnancy was due to the increase of thrombin and plasmin activity. During Caesarean section, it has been noted that increase of FPA in the uterine blood promoted the coagulation activity.

D'Alessandro et al. (1989) studied the changes in human parotid salivary proteins and sialic acid levels during pregnancy. They collected saliva of 107 pregnant women, 9 puerperal and 7 non pregnant controls. No significant changes were found in salivary flow rate, pH and amylase levels. They observed that during pregnancy and the puerperium, the total protein levels were decreased. The sialic acid levels decreased during pregnancy but returned to normal in the puerperium.

Zhang et al. (1993) determined serum pregnancy specific ß-1 glycoprotein (sp1), human placental lactogen (HPL) and urinary estrogen/creatinine (E/C) ratio levels in predicting low birth wt. (LBW) in women with normal pregnancy and patients with LBW. They observed better urinary E/C ratio than that of serum sp1 and HPL and was increased with the reduction of fetal weight. They noted that with the increase of serum sp1 and HPL levels and urinary E/C ratio, the specificity in predicting LBW was decreased. The specificity would increase over 90% when serum sp1 level was less than 50 mg/L, HPL less than 2.0 mg/L and E/C ratio less than 10.

In the current investigations, the serum protein profiles of women in stress situations both during normal delivery and during Caesarean deliveries were analyzed. An interesting finding was that when women were in acute physical stress i.e. pain, there was a discernible reduction in quantities of the 7S globulin fractions. In normal deliveries, the physical stress is before delivery. The protein bands are faint or missing in samples taken before normal delivery whereas these protein bands are restored in protein profiles of samples taken after delivery when women have recovered from the trauma of child birth. In delivery by Caesarean section physical stress was after the delivery. The serum protein profiles of samples taken after Caesarean delivery shows reduction in protein profiles.

These findings clearly demonstrate that factors which cause acute stress or pain elicit a response in the body which causes a rapid depletion of the 7S globulin fraction. It is likely that the trauma of childbirth as well as Caesarean section causes a rapid reduction either in the antibody (globulin fraction) or some other protein fraction whose molecular weight is within that range.

c. Hormones during pregnancy

Serum progesterone, cortisol, thyrotrophin (TSH), Prolactin (PRL), placental lactogen (HPL), estriol, plasma ACTH and plasma CRH levels have been studied in non-pregnant, pregnant and post-partum women. Significant circadian rhythm with a nadir at 8h and acrophase around midnight was found in progesterone levels and an

inverse relation was found in cortisol rhythm in pregnant women (Junkermann et al. 1982, Perez - Lopez et al., 1984). Diurnal pattern was found for plasma ACTH and serum cortisol before and after delivery with lower post partum concentrations but no diurnal changes have been observed with progesterone and plasma CRH after delivery (Allolio et al. 1990). Slightly lower TSH level and slightly higher PRL levels have been observed in pregnant women than in non-pregnant women but no significant changes were observed in HPL and estriol (Perez - Lopez et al. 1984).

Salivary cortisol, progesterone, estriol and estradiol levels have also been studied and no relation was found between either plasma or saliva estriol or progesterone level or the saliva estriol : progesterone ratio in normal pregnant women (Scott et al. 1991). A clear circadian rhythm was observed in salivary cortisol during pregnancy with an increase in 25th to 28th week and highest in late pregnancy, returning rapidly to normal concentration after delivery. Correlation was observed between serum progesterone increase and saliva cortisol increase in late pregnancy (Allolio et al. 1990). A 24 hour hormone secretion in women with preterm labor was studied (Taleb et al. 1993). An estradiol-cortisol shift due to increased cortisol was found. Cortisol circadian secretion was persistent and acrophases at midday were also observed in preterm labor.

Preterm labor can result due to pregnancy induced hypertension, preeclamptia, eclamptia, diabetes, anemia, shock, mental tension etc. when the fetus is not viable and expelled out i e. abortion. In some females there are recurrent abortions.

d. Stress during pregnancy

Stress is a psychological reaction to fear, wonder, shock etc. Stress may be acute or chronic and may affect day to day, emotional status. It affects more in delicate situations like pregnancy. Several studies were conducted to know the changes in the constituents of blood particularly electrolytes, proteins, hormones etc. Anxiety and depression are the variants of stress.

Anxiety state, fears and psychophysiological reactivity have been studied in women with a risk pregnancy as well as in normal pregnant women. It has been found that ultrasound examination and missile attacks makes pregnant women more anxious and insecure. (Skov et al. 1991, Liebermann et al. 1993).

Significantly higher anxiety score before delivery and clear decline of symptoms after delivery was observed in women with a risk pregnancy and in normal pregnant women (Guidozzi et al. 1990) while in contrast, postpartum anxiety and depression was found in pregnant women during the first three months after delivery. (Thalassinos et al. 1990). It has been found that 25% of patients undergoing caesarean delivery were depressed. In contrast vaginally delivering patients were not depressed. (Mathew et al. 1992). Significant increase in hormone levels was found during labor and an increased risk of spontaneous abortion and term low birth weight was noticed for women with high job stress. (Shalev et al. 1985, Brandt et al. 1992).

Pregnancy too is an important contributory factor for depression. Pfost et al (1990) studied the questionnaire completed by women during the 8th month of pregnancy and one month after delivery and observed that the level of postpartum depression depends on marital status, antepartum depressive symptoms and difficulty of pregnancy. They also found that in unmarried mothers, the depressive symptoms persisted after childbirth. Depression increases if the pregnancy is risky or some type of intervention is required. Boyce et al.(1992) studied the increased risk of postnatal depression after emergency caesarean section. They divided 188 women in 3 groups. a) an emergency Caesarean delivery, b) forcep delivery and, c) spontaneous vaginal delivery. A significant difference was found in 3 groups at 3 months postpartum only. They concluded that women who had an emergency Caesarean section had more than 6 times the risk of developing postnatal depression, 3 months postpartum, than women who had spontaneous vaginal or forcep delivery section.

Hannan et al.(1992) studied the relation between early postpartum mood and post-natal depression. They observed that baby delivered by Caesarean with low birth weight, a difficult delivery and bottle feeding was significantly related with a high EPDS

score in the first week post-partum. Bottle feeding and delivery by Caesarean section were the only factors associated with depression at six weeks.

9% women were depressed during pregnancy and 12% women after delivery (O'Hara and Michael). It has been reported that women who experienced postpartum depression had more stressful life events and less support from their spouses than women who did not experience postpartum depression.

Smoking plays a very important role during pregnancy. Kuhnert et al. (1992) measured zinc intake and zinc status in mother and fetus with Atomic Absorption spectroscopy and observed that in the non-smoking parturient both cord vein plasma zinc and cord vein alkaline phosphatase activity are positively related to maternal zinc intake whereas in smoking parturients there was no relationship between zinc intake and fetal zinc status except for a negative relation with cord vein plasma zinc. They also reported that maternal zinc intake is not related to maternal zinc status but to fetal zinc in a normal pregnancy.

There is a quiet discipline in the whole naturally occurring pregnancy. Many variations occurring during pregnancy are in a cyclical manner, called circadian variations. Diurnal, circadian and circannual variations have been studied.

Changes during pregnancy are also reflected in urine and saliva. Kuo et al. (1992) determined 24 hour urinary protein excretion rates. Protein results were compared with hypertensive pregnant women. They concluded that dipstick urine analysis cannot detect the presence of proteinuria in pregnant women. It has been reported that urinary leakage was more in women elder than 30 than women younger than 30 during pregnancy. (Dimpfl et al. (1992). They concluded that vaginal delivery caused permanent stress urinary incontinence (SUI).

5. SUMMARY

a) Women in acute labor pain (who give birth by normal delivery) show depletion in the serum protein 7S globulin fraction. Levels of these proteins are restored after childbirth.

b) There is a reduction in 7S globulin fraction in sera of women after delivery by Caesarean section.

6. REFERENCES

ALLOLIO, B., J.HOFFMANN, E.A. LINTON, W.WINKELMANN, M.KUSCHE and H.M.SCHULTE. 1990. Diurnal salivary cortisol patterns during pregnancy and after delivery : Relationship to plasma corticotrophin - releasing hormone. CLIN. ENDOCRINOL. 33(2) : 279-290.

BARNEA, E.R., M.K. SANYAL, C.BRAMI and P.BISCHOF. 1986. In vitro production of pregnancy asociated plasma protein A by trophoblastic cells. ARCH. GYNECOL.. 237(4): 187-190.

BERSINGER, N.A., L.M. GERRIE, G.LUKE and A.KLOPPER. 1986. Serum concentration of pregnancy specific and pregnancy associated proteins in early gestation. ARCH. GYNECOL. 237(4) : 221-228.

BERSINGER, N.A., H.SCHNEIDER and P.J.KELLER. 1986. Synthesis of placental proteins by the human placenta perfused in vitro-preliminary report. GYNECOL. OBSTET. INVEST. 22(1) : 47-51.

BIEGLMAYER, C., A. FEIKS and R. RUDELSTORFER. 1986. Laminin in pregnancy. GYNECOL. OBSTET. INVEST. 22(1) : 7-11.

BOYCE, P.M. and A.L. TODD. 1992. Increased risk of postnatal depression after emergency caesarean section. MED.J.AUST.157(3) : 172-174.

BRANDT, L.P.A. and C.V. NIELSEN. 1992. Job Stress and adverse outcome of pregnancy. AM. J. EPIDEMIOL 135(3) : 302-311.

CARPANI, G., F.MARINI, L.GHISONI, M.BUSCAGLIA, E SINIGAGLIA AND G.MORONI. 1992. Red cell and plasma ferritin in a group of normal fetuses at different ages of gestation. EUR. J. HAEMATOL. 49(5) : 260-262.

CIMELLARO, M., A.D'ANNA and A.FREGA. 1985 (recd.1986) Peculiarity of serum protein levels in gestosis of third trimster. PATOL. CLIN. OSTET. GINECOL 13(4) : 295-299.

COUSINGS, L., L.RIGG, D.HOLLINGSWORTH, P-MEIS, F.HALBERG, G.BRING and S.S.C. YEN. 1983. Qualitative and quantitative assessment of the circadian rhythm and diurnal excretion of plasma cortisol and urinary free corticoids. AM.J. OBSTET. GYNECOL. 145(4) : 411-416.

D'ALESSANDRO, S., H.M.CURBELO, O.R. TUMILASCI, J.A. TESSLER and A.B.HOUSSARY. 1989. Changes in human patotid salivary protein and sialic acid levels during pregnancy. ARCH. ORAL. BIO. 34(10) : 829-832.

DIMPFL, T., U.HESSE and B.SCHUESSLER. 1992. Incidence and cause of postpartum urinary stress incontinence. EUR.J.OBSTET. GYNECOL. REPORD.BIOL. 43(1) : 29-34.

GERHARD, I., E.KATZER and B.RUNNEBAUM. 1991. The early pregnancy factor (EPF) in pregnancies of women with habitual abortions. EARLY HUM.DEV. 26(2) : 83-92.

GOEL, M., K.DAS, H.P. GUPTA, A.R.CHOWDHARY and V.P. KAMBOJ. 1991. Serum magnesium level in pregnancy. INDIAN VET. MED. J. 15(2) : 83 - 87.

GUIDOZZI, F., M.MACLENNAN, K.M.GRAHAM AND C.P. JOOSTE. 1992. Salivary Calcium, magnesium, phosphate, sodium and potassium in pregnancy and labour. SAMJ. (S.AFR.MED.J.) 81(3) : 152-154.

HALLER, G , P.LINNEKE, P.VOSS and W.JESKE. 1988. Reference ranges of _-fetoprotein (AFP) and pregnancy specific ß, glycoprotein (spl) in early pregnancy. ZENTRALBL. GYNAEKOL. 110(24) : 1556-1560.

HANNAN, PATRICIA, D.ADAMS, A.LEE, V.GLOVER AND M.SANDLER, 1992. Links between early post-parium mood and post-natal depression, BR.J.PHYCHIATRY. 160 (JUNE), 777-780.

HOWELL, R.J.S., A.E. BOLTON and T.CHARD. 1986. Placental protein 14 in late pregnancy. ARCH. GYNECOL. 239 (1) : 27-30.

ILYES, I., J. JEZERNICZKY, J.KOVACS, E. DVORACSEK and S.CSORBA. 1985 (Recd. 1986). Relationship of maternal and newborn serum ferritin concentration measured by immunoradiometry. ACTA. PAEDIATR. HUNG. 26(4):317-322.

JOHNSTONE, R.W., R.J.KELLAR. 1968. Section II, Chapter VIII. Changes In the Maternal Organism consequent upon Impregnation. In : A text book of midwifery. ALLIED Publishers Ltd. 1968. i-xvi, pp 631.

JUNKERMANN, H., H.MANGOLD, P.VECSEI and B.RUNNEBAUM. 1982. Circadian rhythm of serum progesterone levels in human pregnancy and its relation to the rhythm of cortisol. ACTA ENDOCRINOL. 101 (1) : 98 - 104.

KAUFER, M. and E. CASANUEVA. 1990. Relation of pregnancy serum ferritin levels to haemoglobin levels throughout pregnancy. EUR. J. CLIN. NUTR. 44(10) : 709 - 716.

KOU, V.S., G.KOMANTAKIS and E.D.N. GALLERY. 1992. Proteinuria and its assessment in normal and hypertensive pregnancy. AM. J. OBSTET. GYNECOL. 167 (3) : 723 - 728.

KUHNERT, B.R., P.M. KUHNERT, S.L. GROHWARGO, S.WEBSTER, P.ERHARD and N.LAZEBNIK. 1992. Smoking alters the relationship between maternal zinc intake and biochemical indices of fetal zinc status. AM. J. CLIN. NUTR. 55(5) : 981-984.

KURZEL, R.B.. 1991. Serum magnesium levels in pregnancy and preterm labor. AM.J. PERINATOL. 8(2) : 119-127.

LIEBERMAN, D. and S.HAREL. 1993. Anxiety during pregnancy at the time of the Gulf war. PSYCHOL. REP. 72(2) : 600-602.

MAJIMA, T. and M.NAKAI. 1991. Quantitation of new pregnancy -associated protein in pregnancy sera by means of a single radial immunodiffusion technique. JPN. J. FERTIL. STERIL. 36(2) : 202 - 209.

MARTINEZ, M.E., C.SANCHEZ, M.SALINAS, J.PELLEGRINI, A.CARRASCO, P.CATALAN, G.BALAGUER and QUERO. 1986. Ionic Calcium levels during pregnancy, at delivery and in the first hours of life. SCAND. J. CLIN. LAB. INVEST. 46(1) : 27-30.

MATHEW, J.P., L.A. FLEISHER, J A. RINHOUSE, F.B. SEVARINO, R.S. SINATRA, A.H. NELSON, E.K. PROKOP and S.H. ROSENBAON. 1992. ST segment depression during labour and delivery. ANESTHESIOLOGY. 77(4) : 635 - 641.

MUCHMORE, A.V. . 1986. Uromodulin : An immunoregulatory glycoprotein isolated from pregnancy urine that binds to and regulates the activity of interleukin-1. AM.J. REPROD. IMMUNOL. MICROBIOL. 11 (3) : 89-93.

O'HARA, M.W.. 1986. Social support, life events and depression during pregnancy and the puerperium. ARCH. GEN. PSYCHIATRY. 43(6) : 569 - 573.

PEREZ-LOPEZ, F.R., F.AISA, M.URACIS, M.F.MARTINEZ CASMAYOR and M.P. FARJAS. 1984. Circadian oscillations of thyrotropin, prolactin, cortisol, placental lactogen and estriol in late human pregnancy. NEUROENDOCRIONOL. LETT. 6(4) : 231-242.

PFOST, K.S., M.J.STEVENS and C.U.LUM. 1990. The relationship of demographic variables, antepartum depression and stress to postpertum depression J.CLIN. PSYCHOL. 46(5) ; 588 - 592.

RAMAN, L., P. YASHODHARA AND L.A. RAMARAJU. 1991. Calcium and magnesium in pregnancy. NUTR. RES. 11(11) : 1231-1236.

SCOTT, E.M., A.THOMAS, H.H. G. MAGARRIGLE and G.C.L.LACHELIN. 1991. A comparison of serial ultrasound measurements of fetal adrenal glands with maternal plasma and saliva oestriol and progesterone levels in normal pregnancy. J.OBSTET. GYNAECOL. (BASINGSTOKE)11 (6): 381-385.

SHALEV. E., A.ERAN, S.HARPAZKERPEL and H.ZUCKERMAN. 1985. Psychological stress in women during fetal monitoring (hormonal profile). ACTA. OBSTET. GYNECOL. SCAND. 64(5) : 417 - 420.

SHARMA, R., K.C. SHINGHAL, K.TEWARJ and M.GUPTA. 1991. Serum zinc levels in nonpregnant and pregnant women. INDIAN J. PHARMACOL. 23(4) : 242 - 246.

SKOV, R.V.. 1991. The attitudes of pregnant women to ultrasound screening. A questionnaire investigation. UGESKRLAEG. 153(4) : 283 - 284.

SUMIOKI, H., H.SHIMOKAWA, S.MIYAMOTO, K.UEZONO, T.UTSUNOMIYA and H.NAKANO. 1989. Ciradian variations of plasma atrial natriuretic peptide in 4 types of hypertensive disorder during pregnancy. BR. J. OBSTET. GYNAECOL. 96 (8) : 922 - 927.

TALEB, J., B.KRAUSE and G.GOERETZLEHNER. 1993. Twenty four hours hormone secretion profiles of cortisol and estradiol. HORM. METAB.RES. 25(8) : 442-443.

TEI, A., S.MUTOH, Y.YAOI and M.SAITO. 1989. Serum levels of coagulation - fibrinolysis factors in normal pregnancy, labor and puerperium and in caesarean section delivery. BULL TOKYO MED. DENT. UNIV. 36(2) : 19-28.

TEWKSBURY, D.A., E.S. TRYON, R.E. BURRILL AND R.A. DART. 1986. High molecular weight angiotensinogen. A pregnancy associated protein. CLIN. CHIM. ACTA. 158(1) : 7-12.

THALASSINOS. M., C.ZITTOUN, F.ROUILLON and P. ENGELMANN. 1993. Post partum anxiety and depression in pregnant women. J.GYNECOL. OBSTET.BIOL. REPROD. 22(1) : 101-106.

ZHANG, W. Y., TENG, HONG, WNAG, LI-YAN and YAN, GUO-LAI. 1993. Serum Sp1., HPL levels and urinary estrogen/creatinine ratio in the detection of low birth weight. CHIN. MED. J. 106(6) : 437-440.

ZHOU, Y., L.SHIMIN, D.YUXIA AND W.LIN. 1991. The relationship of serum calcium, magnesium, zinc and copper between maternal and cord blood. ACTA. NUTR. SIN. 13(1) : 58 - 62.

D. CHAPTER 3

Variation in Serum Proteins of
(A) Students in Stress Situations and
(B) Patients of Depression.

1. PREAMBLE

There are several mental processes by virtue of which a person is protected from mental and psychological agitation and his misery reduces viz. repression, denial, regression, projection, displacement, rationalization, sublimation, identification.

Repression is the exclusion from awareness of memories, emotions/impulses that would cause anxiety and distress if allowed to enter consciousness. Denial is believed to be employed when persons behave as though unaware of something that they might reasonably be expected to know. Regression is the unconscious adoption of patterns of behavior appropriate to an earlier stage of development. Projection involves the unconscious attribution to another person of thoughts or feelings that are in fact one's own. Displacement is of paramount importance. Displacement involves the transferring of emotion from a situation or object with which it is properly associated to another that gives less distress.

If there is any type of irregularity or dysfunction in the defenses, there will be loss of the normal functional activities and the person becomes psychologically disturbed. There is a normal range. Which is subjective. But when the person's psychological agitation goes beyond a particular limit, there is manifestation of psychological symptoms and we call him a psychic patient.

There are various factors which cause the changes in the normal pattern of the psychic setup.

i) **Biological factors** : Attempts to establish a biological basis have run the familiar gamut from genetic and constitutional factors though neurophysiological and biochemical alterations as well as implicating various related considerations such as sleep disturbances.

Hereditary predisposition : The precise role of heredity in this disorder is far from clear, although, it seems realistic to consider it as an important interactional factor in the total picture.

Neurophysiological factor : It is possible that imbalances in excitatory and inhibitory processes may predispose some people towards extreme mood swings.

Biochemical Factors : Evidence suggests that the catecholamine function may be decreased in depression and increased in mania. It may be noted that various mental disorders including manic depressive psychoses may lead to alterations in brain biochemistry rather than the other way around.

ii) Psychological and interpersonal factors : Depressive patients show an unusually rigid conscience development, which prevent the overt expression of hostile feelings and makes them particularly prone to feelings of guilt and self blame when things go wrong.

Severe stress : Typical participating stresses in severe depressive reactions fall into 3 general categories

(a) death of a loved one

(b) failure in an interpersonal relationship, usually with one's spouse or

(c) a severe setback or disappointment in the work or other goals to which an individual has been devoted. All of these participating conditions involve the loss of something that has been of great value to the individual.

Feelings of helplessness and loss of hope.

Social roles and communication : The depressive tends to adopt a role that places others in the position of supporting and caring for him. The depressive generally does obtain some secondary gains from his symptoms.

There are several persons in the community who have the potential changes in the structural or functional psychological setup. But there is no manifestation. But at certain point, the person becomes symptomatic. These factors are the precipitating factors which are physical, psychological and social. The physical factors are hypothyroidism, tumors, metabolic disorders, steroid therapy etc. The psychological factors are loss of self-esteem due to a set-back or misfortune. Social factors include the moving house, job, difficulties and family disturbances. Perpetuating factors or aggravating factors include the factors which prolong the course of the disease. The psychiatric disorder leads to secondary demoralization and the demoralization leads to increase in the intensity and severity of the disorder.

Classification of the psychiatric disorder is based on the causative factors, presentation and prognosis. Most common diseases are the neurosis like anxiety neurosis, obsessive-compulsive disorders, dissociative disorders like hysteria, hypochondriasis, stress adjustment reactions, etc. Psychoses are severe forms of psychological disorders. The main disorders are schizophrenia and affective disorders. The affective disorders include mania, depression and mania-depression. In rare diseases, there are organic disorders like Delirium, Dementia, Amnestic syndrome etc. Various types of disorders seen in the normal population like personality disorders, alcohol abuse and dependence, drug abuse and dependence, eating disorders, psychosexual disorders etc.

Depression

Depression is classically divided into endogenous depression and relative depression. Criteria arranged for endogenous D are prevention and unresponsive depression i.e. feeling sad and low in spirit, rarely morning waking, diurnal variation of mood,

profoundly depressive ideas i.e. guilt and suicidal thoughts, lack of an obvious precipitating cause and the stable premorbid personality. The criteria for reactive depression are, a fluctuating depression; irresponsive to environmental changes, self pity rather than self blame, a clear precipitating cause, a vulnerable personality and absence of criteria for endogenous depression. Individuals who satisfy the above criteria are considered as cases of depression.

Environmental atmosphere has a great impact over affective disorders like loss of mother before age of 11 yrs, 3 or more children under 14 yrs. of age living at home, lack of confiding relations, lack of employment etc. Physical illnesses contribute to depression particularly cancer, heart disease, viral illness, Cushing's syndrome, Addison's disease, hypothyroidism, corticosteroid therapy, antihypertensive drugs. Negative attitude to oneself towards outside world and towards future is the most important precipitating factor in case of depression.

The depressed patients have depressed, miserable and unhappy mood. They talk slowly, monotonously and the topic is often incomplete. They lack energy and are retarded and apathetic. They always feel futile, repent and have a guilty feeling. They think themselves unworthy for the community. Their verbal memory is impaired. Several physical symptoms such as early waking, loss of appetite, loss of weight, constipation, loss of libido, fatigue etc. may be seen. Their behavior is agitated or retarded. Auditory hallucinations is an important signal of depression.

Treatment :

For treating such patients, varieties of treatments are practiced according to the personality, environment and if possible according to the cause of the depression. In physical treatment the patient is given tricyclic and related antidepressants such as imipramine and amylotryptiline. These drugs potentate actions of monoamines, noradrenaline and serotonin by inhibiting their reuptake into the nerve terminals. Patients with established heart disease are given meanserine. Serotonin uptake inhibitors, fluvoximine can be given.

Physical treatment is not sufficient, supportive treatment is given in the form of cognitive therapy. Cognitive therapy aims at modifying patterns of thinking in a positive way. Electroconvulsive therapy (ECT) is indicated when the risk of suicide is so great that one cannot wait for the delayed therapeutic effect and antidepressant drug.

In the overall treatment program it is important to deal also with unusual stresses in the patients life, since an unfavorable situation may lead to the recurrence of the reaction as well as necessitating longer treatment.

The mortality rate for depressive patients appears to be about twice as high as that for the general population. However, because of the higher incidence of suicide to recover, there is requirement of 4 to 18 months. 15 to 20 % of depressives never fully recover.

Mania

Mania is subjective feelings of high spirits. In the manic patients the mood is elevated infectious and labile. They talk fast, pressurized and the talkings are full of ideas. They are restless and easily distractable. They are highly enthusiastic and self confident. They always have significant religious influence acting over them. They have complaints of sleeplessness and mild to moderate weight loss. They are interested in hypersexual activities and they prefer to drink alcohol excessively. Visual hallucinations is the important feature of Mania.

Mania and depression, are two sides of the same coin. The causes of depression are the same as that of mania but it is not obligatory that the person suffering from depression must have mania or vice versa. Pure depression or pure mania are also observed. These are called as unipolar affective disorders. Pure depression is more common than pure mania. When both are existing in a rhythmical manner, then the disorder is called as bipolar affective psychosis.

The Bipolar depression must be differentiated from other psychological disorders which have a somewhat similar type of symptom spectrum. The diseases are drug induced psychosis, acute schizophrenia, dementia, hyperthyroidism etc.

Treatment :

Treatment of mania includes drug treatment, social and psychological treatment as well as ECT. In acute attack, neuroleptic drugs such as haloperidol, chorpromarine are given. Lithium carbonate or Lithium citrate are given prophylactically. Recently, carbamazitine has been used as an alternative prophylactic agent. Virtually all manic patients recover but the main problem is how to prevent relapse. 5 to 10% develop chronic debility that may follow the first episode 25% have affective symptoms life long.

Anxiety

Anxiety is a natural reaction in the phase of danger. It becomes morbid when symptoms are cut of proportion to external circumstances. However, there is no clear distinction between the features of normal and pathological anxiety. Prevalence of anxiety in a community is 2 to 5%. Women are more susceptible than men. The patients with generalized anxiety have tremors, sweating, palpitations, chest pain breathlessness, headache, dizziness, diarrhea, increased frequency of micturition, initial insomnia and poor concentration. There are psychological symptoms like apprehension, fears of impending disasters, irritability and depersonalization.

There are some specialized anxieties like phobic anxiety in which there is phobia for the things surrounding and panic anxiety in which there are recurrent attacks of acute onset anxiety.

Treatment :

The main treatment of anxiety is psychological, but in severe conditions physical treatment by drugs such as benzodiazapines (diazapalm, nitrazapalm etc.) and

propranolol is given. In the psychological treatment reassurance, relaxation training by exercise such as yoga, behavioral therapy such as desensitization and flooding of program practice, group based psychotherapy etc. are included. The treatment of the disease is done by anxiolytic drugs, antidepressants and psychosurgery (tractonomy) and limbic leukotomy. 2/3rd cases improve with in one year.

Schizophrenia

Schizophrenia means rending or splitting of psychic functions, in which the normal integration of emotional and congnitive functions are ruptured. Annual prevalence 2 to 4 / 1000 / year. Life time risk is 1%. Higher rates in Yugoslavia and Tamils in south Indians are observed. (From : Lewis et al. 1994).

2. INTRODUCTION

A review of the literature reveals that stress, anxiety and depression, have been studied extensively. Evans et al. (1994) studied stress, arousal, cortisol and secretory immunoglobulin A in students undergoing assessment and found that all measures were higher on the day of assessment. Stress, anxiety and depression was studied in a group of senior health service staff by Caplan (1994). Circadian changes of body temperature, circadian rhythms in cortisol and prolactin have also been studied. (Tosca et al. 1982). Ellcot et al. (1990) studied the impact of life stress on the course of bipolar disorder in 61 outpatients for 2 years. They noted a significant relationship between life events and relapses or recurrence of disorder.

In all these studies psychological and biochemical variables were studied on a number of subjects of depression. However, serum protein profiles have not been studied in students.

The aim of this study was to analyze the serum protein profiles of students who were in acute stress i.e. just before their crucial examination and subsequently in a relaxed state i.e. 2 days after completion of their examination.

Serum protein profiles of known cases of depression were also analyzed by PAGE. The present chapter also includes absolute counts of CD_{57} Natural Killer cells in peripheral blood of 8 depressed patients.

In order to find whether there is an increase in the incidence of patients reporting for treatment of depression, specifically on certain lunar days, studies were carried out on the number of patients reporting for treatment during one complete lunar cycle.

3. MATERIAL AND METHODS

A. STUDENTS APPEARING FOR A CRUCIAL EXAMINATION.

Blood samples of Ist M.B.B.S students of Government Medical College, Nagpur were collected from seven students one day prior to their final examination and 2-3 days after completion of their examination. Samples were centrifuged and the serum proteins were analyzed by PAGE as described in Chapter I. For subject no. 3, it was his third attempt to pass the examination and hence was in great stress since failure meant that he would be debarred for pursuing a career in medicine.

B. PATIENTS OF DEPRESSION

Blood samples of 9 patients of depression (Table III.1) were collected from E.S.I.S Hospital, Nagpur. Serum proteins were analyzed by PAGE. CD57 / HNK - 1 (VC 1.1, dilution 100), antibody used was from Sigma chemicals. Absolute counts of CD57 +ve Natural Killer cells of 8 patients of depression were studied by immunocytochemistry as described in Chapter I.

Data on number of patients reporting for treatment of depression for 1 lunar cycle was also collected and the number of patients reporting for treatment on different lunar days was analyzed. (π 2.)

Table III.1 - Age, Sex, Symptoms and Treatment of Patients of depression (1 to 9) whose serum samples were analysed by PAGE.

S.No.	Sex	Age	Symptoms	Treatment
1.	F	35	Giddiness, insomnia	Meitriptiline since 2 yrs.
2.	M	40	Giddiness, insomnia, loss of appetite, loss of interest in life, does not atted work.	Diazepam
3.	F	35	Giddiness, irritability, chest pain.	Diazepam. Amphiresparin.
4.	M	36	Does not attend job since 3 months, first consaltation.	Meitriptiline.
5.	F	40	Frightened, cannot bare too much of joy or unhappiness.	T.Diazepam 5mg.. T.Depsonil 25mg.
6.	M	38	Giddiness, frightened, does not attend job since 4-5 months.	T.Alprazolam 25mg.. T.Depsonil 25mg.
7.	F	55	Giddiness, lethargy, quarreling, was admitted several times.	T.Diazepam 5mg., T.Depsonil 25mg.
8.	M	43	Giddiness, insomnia, loss of appetite, does not attend since 10 months.	T.Alprazolam 25mg.. T.Depsonil 25mg.
9.	F	27	Irritability, chest pain, quarrels at home lead to these symptoms.	T.Diazepam 5mg.. T.Depsonil 25mg.

Table III.2 Number of patients reporting for treatment of depression during one Lunar Cycle.

Date	Lunar day	No. of Patients	M	F
7-4-97	30	18	11	7
8-4-97	1	Holiday		
9-4-97	2	25	14	11
10-4-97	3	9	5	4
11-4-97	4	13	8	5
12-4-97	5	11	3	8
13-4-97	6	Holiday		
14-4-97	7	Holiday		
15-4-97	8	13	6	7
16-4-97	9	Holiday		
17-4-97	10	33	21	12
18-4-97	11	Holiday		
19-4-97	12	25	11	14
20-4-97	13	Holiday		
21-4-97	14	15	5	10
22-4-97	15	12	6	6
23-4-97	16	31	12	19
24-4-97	17	14	6	8
25-4-97	18	11	7	4
26-4-97	19	8	3	5
27-4-97	20	Holiday		
28-4-97	21	12	6	6
29-4-97	22	14	8	6
30-4-97	23	25	14	11
1-5-97	24	Holiday		
2-5-97	25	15	4	11
3-5-97	26	25	13	12
4-5-97	27	Holiday		
5-5-97	28	14	8	6
6-5-97	30	17	7	10

4. OBSERVATIONS

A. SERUM PROTEIN PROFILES OF STUDENTS APPEARING FOR A CRUCIAL EXAMINATION.

Fig. 3.1 and fig. 3.2 is a comparison of the serum protein profiles of students one day prior to the commencement of their Ist M.B.B.S. examination and 3 days after the completion of their examination. It was noticed that while prealbumin, albumin and transferrin bands were present in both serum samples, variations were seen in post transferrin bands. The 7S globulin fraction located beyond C_1 shows depletion in the protein content to an extent that bands are not detectable under stress situations i.e. prior to the examination but their levels are restored after the subjects are back to their normal relaxed state.

In subject 1, considerable depletion of proteins is noticed prior to the examination (1B). After the examination, the C_1 band can be clearly seen while the other bands are faint.

In subjects 2, 4 and 6 the globulin fraction appears very faint and the post transferrin bands are not visible in serum samples of students, prior to their examination as compared to their serum samples after the examination.

An interesting finding was that in the serum of subject 3 before appearing for the examination (3B), the post transferrin bands are missing altogether whereas these bands are restored when the subject is back to his normal relaxed state. Of the 7 subjects examined, the result of subject no. 5, were contrary to those of others. In his serum, the post transferrin bands were missing in the serum sample after the examination.

In general, it can be said that students under stress exhibit a deficiency in the 7S serum protein fraction.

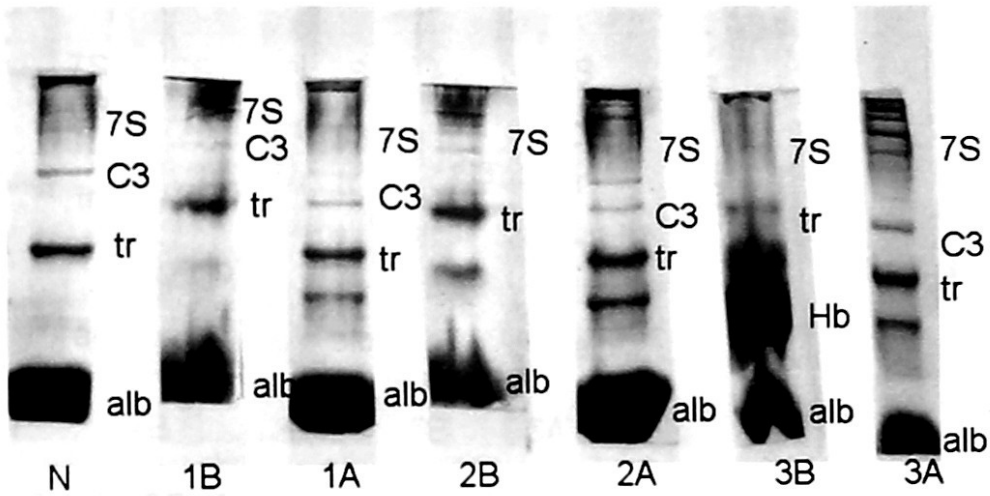


Fig. 3.1 PAGE of serum proteins of students before (B) and after (A) appearing for a crucial examination.

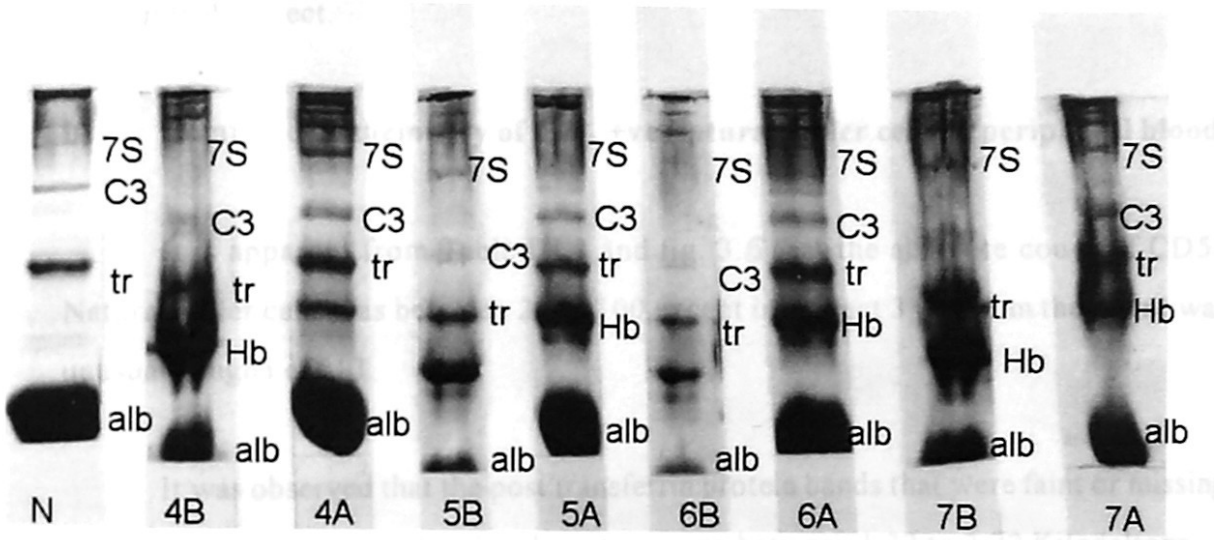


Fig.3.2 PAGE of serum proteins of students before (B) and after (A) appearing for a crucial examination.

B. OBSERVATIONS ON PATIENTS OF DEPRESSION

I) Serum protein profiles :

a) Fig. 3.3 and fig. 3.4 illustrates the PAGE of 6 µl serum protein samples of 9 subjects of depression (Table III.1). Their protein profiles indicate that except for subject 01 in whom there is a general hypoproteinemia with reduction in intensity of all the bands, not much difference in protein profiles was observed in the other 8 subjects and their protein profiles did not vary much from the protein profile of a normal healthy (N) control subject.

b) . Immunocytochemistry of CD_{57} +ve Natural Killer cells in peripheral blood.

It is apparent from Table III.3 and fig. 3.5 that the absolute count of CD57 Natural Killer cells was between 20 to 100 except in subject 3 in whom the count was unusually high i.e. 513.

It was observed that the post transferrin protein bands that were faint or missing under stress situations had molecular wt. ranging between 1.32 to 2.72 Kilodaltons.

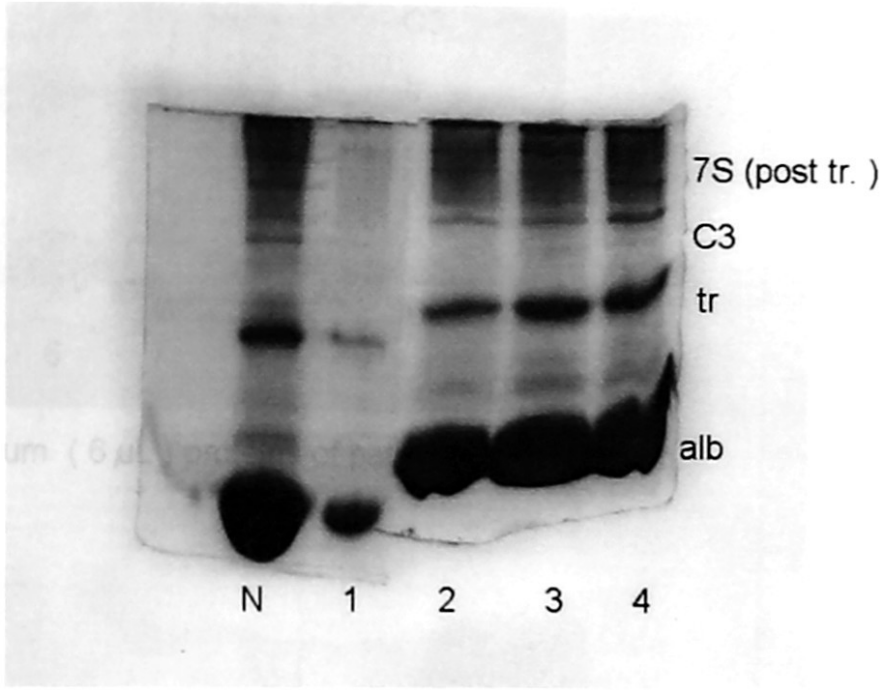


Fig. 3.3 PAGE of serum (6 µL) proteins of patients of depression.

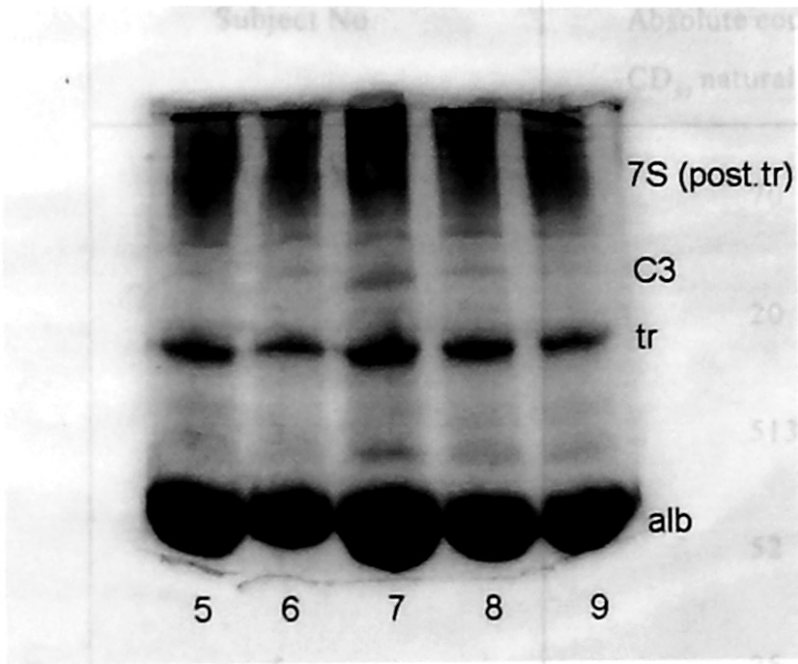


Fig. 3.4 PAGE of serum (6 μL) proteins of patients of depression.

Table III.3 Absolute count of CD_{57} natural killer cells in 1 µl blood of 8 patients (1 - 8) of depression.

Subject No.	Absolute count of CD_{57} natural killer cells
1	70
2	20
3	513
4	52
5	25
6	31
7	23
8	107

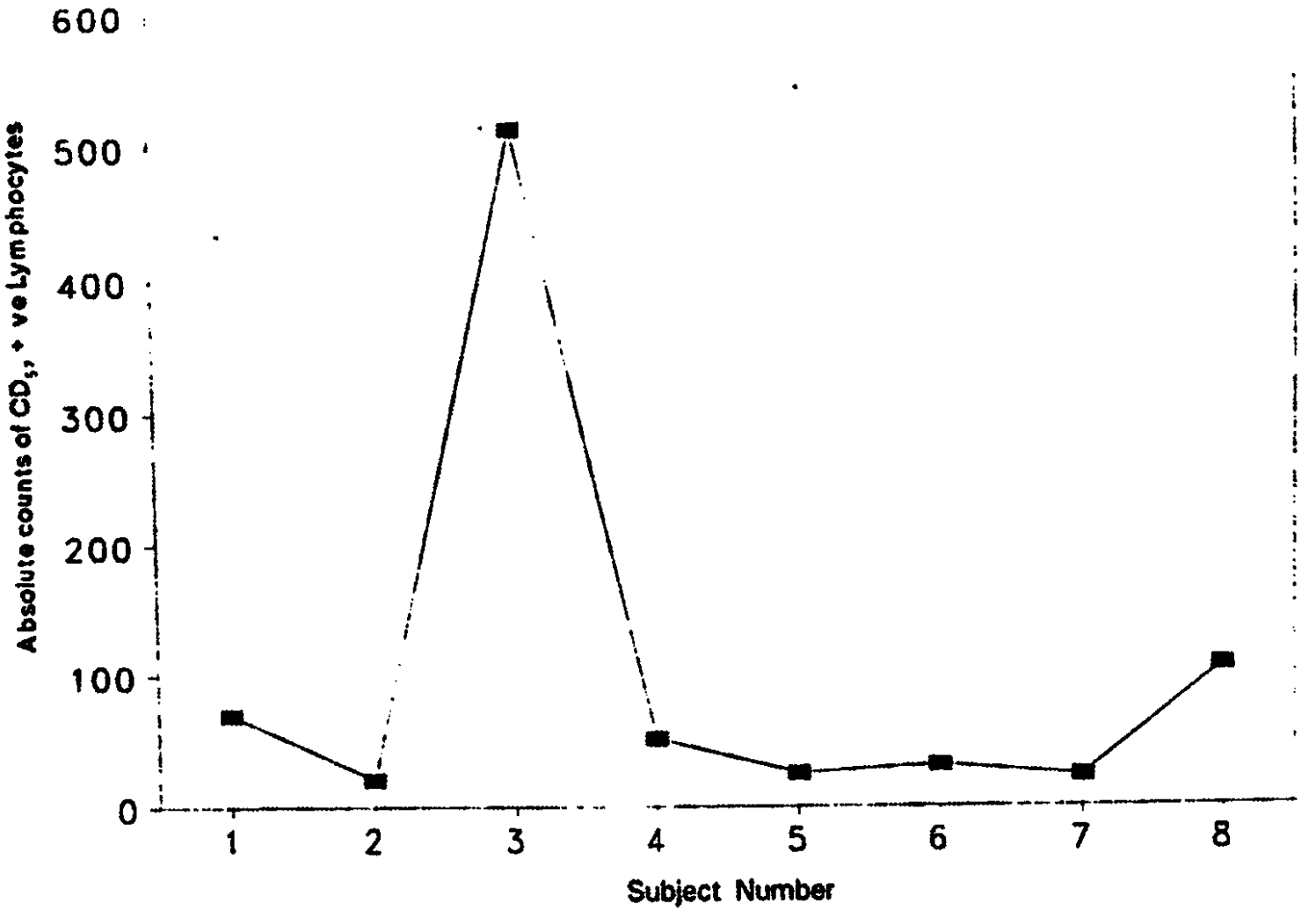


Fig. 3.5 Absolute count of CD57 positive Lymphocytes in 1μl blood of 8 patients (1 - 8) of depression.

II) Lunar cycle studies on patients reporting for treatment of depression

From fig. 3.5 and table III.2 it is apparent that the number of patients reporting for treatment of depression are maximum on lunar days NM (n=18); day 2 (n=25); day 10 (n=33); day 12 (n=25); day 16 (n=31); day 23 (n=25); day 26 (n=25) i.e. 5 to 6 days after full moon and 5 to 6 days after new moon. On lunar day 3 to day 8, the number of patients reporting for treatment are about ½ of the maximum. Lunar days 1, 6, 7, 9, 11, 13, 20, 24, 27 were holidays (H) and hence no patient reported for treatment.

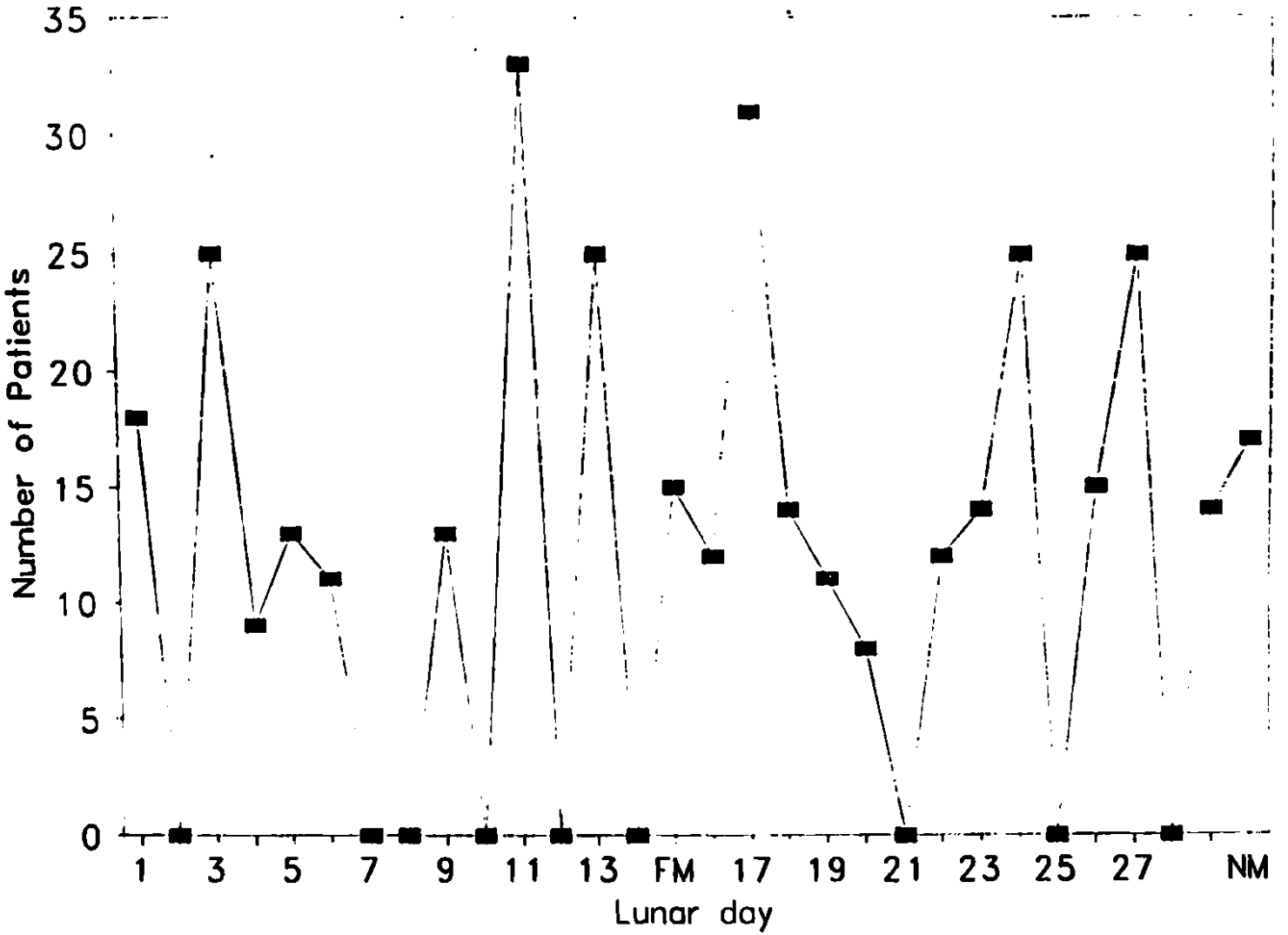


Fig. 3.6 Number of Patients reporting for treatment of depression during one lunar cycle.

5. DISCUSSION

a) Effect of stress in students and children.

Enderson et al. (1991) studied the effect of stress and psychological parameters on human concentration of IgM and complement component C3. They observed that the plasma levels of IgM and C3 was increased. They found a significant correlationship between endocrine and immunological parameters and also between psychological and immunological parameters. They concluded that during acute stress period, the immunological functions were influenced by psychological stress both indirectly and directly.

Tolor and Murphy (1985) studied stress and depression in 285 high school students over a six month interval. They found that on both test administrations, girls displayed a significant relationship between stress and depression. This shows that depression is more common in girls than boys.

Rosenthal et al.(1986) studied depression in hospitalized preschool children with suicidal thoughts and behaviors and compared them with suicidal and behavioral disorders in outpatient preschoolers. Increase in depressed mood, weepiness and parental psychopathology was observed in hospitalized patients than outpatient preschoolers.

Zoccolino et al.(1986) studied 304 first and second year medical students and observed three times greater depression than in the general population. It has been observed that medical school was not the only criterion for depression and depression was common among other students also. A particular group which is persistently under tension is more vulnerable for depression.

Firth (1986) studied levels and sources of stress in 318 medical students in their fourth year. They observed higher mean scores than those in other groups within the general population. No differences in prevalence or in mean scores of stress between

the sexes was observed. Twelve (4%) students reported high intake of alcohol and almost half of the students had increased their intake in the past two years. Relationships with consultants raise the strongest negative feelings, with 102 (34%) students finding these particularly stressful. Stress among medical students should be acknowledged and attempts made to alleviate it.

Taking into consideration the above findings i.e. occurrence depression among medical students, our findings of decreased levels of specific serum proteins in the 7S globulin fraction (fig. 3.1 and fig. 3.2) in medical students appearing for a crucial examination is significant and clearly indicates a direct correlation between these proteins and stress.

Lester and Daved (1990) studied depression and suicide in college students and in adolescents. They found 3.7% college students were seriously depressed and 22% had suicidal preoccupation and 18% of high school students were found seriously depressed and 35% had current suicidal preoccupation. Duggan et al.(1991) studied suicidal behavior in 89 depressed patients. They reported that severe dysphoria, past alcoholism and chronic physical illness were most predictive of suicidal behavior. They observed that different aspects of suicidal behavior had different variables like frequency, degree of intent and severity of medical threat.

Extreme defenses : In case of depression, the person apparently gains some relief from his intolerable stress situations by admitting defeat and giving up light. Since the depressive tends to blame himself for his difficulties he often goes over his past with a microscope, picking out any possible sins of omissions or commission and exaggerating their importance in relation to his present difficulties. Apparently normal grief and depression may be intensified to a pathological degree if the individual feels guilty about having had strong feelings of hostility towards the deceased and or some responsibility for the loved one's death.

Psychological agitation may attain such levels that the child or the adolescent may develop suicidal tendencies. Friedman et al. (1987) studied suicidal behavior in

300 high school students. They reported that 60% of the students thought of killing themselves, 9% had actually made at least one attempt to kill themselves and over half of the attempts reached the attention of mental health professionals.

b) Studies on patients of depression

Kuhs et al. (1992) studied psychomotor activity in unipolar and bipolar depressive patients (25 unipolar and 12 bipolar). They found that the mean activity level of unipolar depression was higher than that of bipolar depressives. There was weak but significant negative correlation between psychomotor activity and self rated sleep time. They found that younger male depressive patients display smaller psychomotor activity than elderly female patients. The psychomotor activity of unipolar and bipolar depression was virtually identical after watching for age and gender.

Matussek et al. 1986 studied the partnership between different types of depression like endogenous bipolar, endogenous unipolar, unclassified depressives and control persons. Some common factors and some differences due to sex were observed. They stated that bipolar depressives had desire for closeness and for love and affection, but unipolar were arrogant, indirect aggressive and emotional. Many conflicts, dissatisfied and demanding nature observed in neurotic and unclassified depressives showed extreme fluctuations.

On the basis of biochemistry, several experiments have been carried out. Plug et al. (1982) studied the circadian course of body temperature and the excretion of methoxyhydroxy phenol glycol (MHPG) and vanillyl mandelic acid (VMA) in a 38 year old woman with bipolar depression. They studied variations for ten days during depression and 10 days during the symptom free interval. They observed a shorter circadian periodicity of MHPG excretion than the periodicity of body temperature and VMA excretion during depression. They also observed that during the symptom free interval the circadian periodicity of all parameters was 24 hours.

The endogenous and non-endogenous depressions can be differentiated biochemically. Tosca et al. (1982) determined cortisol and prolactin circadian rhythms in depressed patients. They classified them as endogenous and non endogenous patients. Significant differences were observed in cortisol and prolactin biorhythms when compared with control subjects. Significantly, lower cortisol suppression percentage was found in the endogenous than in the non endogenous patients and the difference was abolished by the treatment of Benzodiazepins.

One of the prominent features of endogenous depression is early morning waking. Clerk et al. (1989) examined the circadian rhythm of positive and negative affect. It has been observed that all components of positive affect rose from early morning until noon, remained constant until 9 p.m. and then fell rapidly. They found individual differences and clearly defined different patterns in morning and evening types. A link between the mood pattern was obtained. Changes were also studied during the early morning hours (Kenardy et al. (1992). They determined psychological precursors to panic attacks and measured anxiety, threat control, prediction of panic and symptoms during waking hours for one week and concluded that the only significant precursor to panic attacks was the prediction of panic attacks. It has been suggested that anxiety levels follow a circadian pattern.

Wessmann et al.(1992) studied psychiatric disorder in 174 offsprings at high and low risk for depression. They observed suicidal cases, onsets of depression and anxiety disorders in offsprings of depressed patients. Conduct disorder and substance abuse in the subclinical stage were likely to become cases of the same disorder in the following two years in offspring of parents with major depression as compared to asymptomatic offspring. They reported that over 50% of the offspring of depressed patients reported a major depression by age 20.

Souetere et al. (1988) compared the circadian rhythm of body temperature, plasma cortisol, norepinephrine (NE), thyroid stimulating hormone (TSH) and melatonin in 16 endogenously depressed, 15 recovered and 16 normal subjects. Blunting of

the amplitude of these parameters in depressed patients and restoration of the amplitude on recovery was observed. This shows that there is some role of these substances in the etiology of depression. Amines particularly are thought to be closely related to depression.

Roy et al.(1986) compared relative activity of metabolic pathways for nor-epinephrine in 13 endogenous depression patients with 25 normal controls. Higher ratio of urinary excretion of nor-epinephrine and nor-metanephrine to either the sum of two urinary metabolites (3 methoxy 4 hydroxy phenyl glycol + vanillyl mandelic acid) or to the sum of urinary norepinephrine and all of its metabolites was observed. They suggested that there was a shift in endogenous depression to extraneural metabolic pathway for norepinephrine and its metabolites. They therefore concluded that there must be a prominent role of amines in the etiology of depression.

Healy et al. (1986) noticed a circadian rhythm in uptake of 5 hydroxy - tryptamine (5 - HT) which was found in control subjects but was absent in depressives. They observed that endogenous depressives showed lowering of the platelet uptake rates but a difference was not present after clinical recovery.

Endocrine function is also disturbed in depression. Higher cortisol levels were observed during depression and days immediately preceding depressive episodes and in severe manic episodes in contrast with decreased cortisol levels in the early stages of mania (Joyce et al. 1987). Galard et al. (1991) compared salivary cortisol and plasma ACTH of non endogenous and endogenous depressed patients with healthy volunteers before and after administration of 1 mg of dexamethasone. They found that predexamethasone salivary cortisol concentrations were significantly higher in the group with endogenous depression. They noticed a significant correlation between plasma ACTH and predexamethasone salivary cortisol levels in non-endogenous depression patients. They concluded that salivary cortisol substituted for the plasma cortisol in clinical studies and the lack of correlation between ACTH and cortisol levels in saliva of endogenously depressed patients indicates a disturbance in the regulation of cortisol secretion in major depression.

The influence of the time of administration of dexamethasone 0.25 mg. on cortisol secretion was studied by Schulz et al. (1986). Significant decreased level of cortisol in plasma was observed on the next day. Differences in cortisol reaction to controllable and uncontrollable stress and its relationship to nontreated, acutely depressed and treated patients and control group was studied by Cross et al. (1993). They collected saliva cortisol samples and observed that in control patients cortisol was increased after uncontrollable stress and decreased after controllable stress. In acutely depressed patients and in the treated group they observed that cortisol was decreased after both conditions.

Rhythms of blood serotonin and serum melatonin and light therapy in healthy subjects and in patients with nonseasonal depression was studied by Rao et al. (1992) A consistent increase in blood serotonin in patients with nonseasonal depression and healthy subjects after bright and dim light treatment was noticed. They also observed that there was no significant change in melatonin levels of patients and subjects. Bright light, however, caused a 50% decrease in Hamilton rating scale for depression. Moreover, Branchey et al (1982) studied patterns of melatonin and cortisol secretion in depressed patients. They observed that in 3 patients, the hormones were determined simultaneously while in two subjects there were alterations in the circadian rhythms of both hormones during illness. They also noted that the melatonin rhythm remained altered but the cortisol rhythm was normalized. A patient showed a nocturnal melatonin rise and day-night melatonin differences but altered cortisol secretory pattern during depression which was normalized after recovery.

Plasma growth hormone (GH), arderenocorticotrophin (ACTH) and cortisol in unipolar and bipolar depressives were measured by Linkowski et al.(1988). It was observed that, during daytime, depressed patients secrete more GH, and hypercortisolism was evident throughout the 24 hours span. Nadir of ACTH and cortisol rhythms was advanced by an average of 3 hours and concluded that pronounced and consistent abnormalities were found more in patients with unipolar than bipolar depression. Voderholzer et al.(1993) studied the abnormalities of both, the spontaneous and the stimulated release of growth hormone (GH) in patients with endogenous depression.

They compared 6 unmedicated endogenous depressives with 6 age matched healthy men and determined levels of GH at 15 minutes, intervals over 26 hours. Due to a significantly diminished sleep related GH secretion, significantly lower GH secretion was observed in the depressed patients than in the healthy control subjects. They also noted lower GH stimulation following releasing hormones in depressed patients than unhealthy subjects.

An interesting finding is that of Berger et al. (1991) who observed gender differences in alcohol consumption of nondepressed and depressed subjects. They noticed that depressed subjects consume more alcohol than nondepressed and depressed men drank more alcohol per sip than depressed women. After drinking, depression and anxiety decreased. Less consumption of alcohol increased in positive mood in nondepressed women and there was a reduction in tension after alcohol consumption in nondepressed women and depressed men which led them to propose a hypothesis that tension reduction was gender specific.

Marital status is also a causative factor for depression. Smolen et al. (1986) compared couples reporting high and low levels of marital interactions and indicated that marital dissatisfaction and depression in husbands and marital dissatisfaction in wives were associated with high frequencies of right infringing behavior. Both spouses reported pronounced dissatisfaction when the other was transgressive.

In the present investigations, the serum protein profiles of 9 patients of depression were studied. It is intriguing that no differences were observed in their protein profiles as compared to those of normal healthy control subjects. It is probable that since none of the subjects of study were in active stress at the time when blood samples were drawn, the effect of stress was not reflected in their protein profiles. These findings must be viewed in the light of the fact that all the subjects were undergoing medication and were taking antidepressants (Table III.1). Drugs may be responsible for restoration of their normal protein profiles.

In the present findings, quarreling with spouse has been reported to be a major precipitating factor in aggravation of depression, which includes giddiness and insomnia (Table III.1).

Siedel et al.(1996) studied increased CD56+ Natural Killer cells and related cytokines in major depression inpatients by flowcytometry to assess related lymphokines interleukin-2 and interferon Y in a whole blood assay with phytohaemagglutinin (PHA). Significant higher counts of the fraction of CD56+ Natural Killer cells and a greater lymphokine response to PHA was observed in depressed patients than in controls. they also found a significant correlation between lymphokine section and CD56+ cell counts in patients with acute clinical stage but not in healthy controls or inpatients after clinical improvement.

In the present investigations, the absolute counts of CD57 Natural Killer cells of 8 patients of depression were studied. The counts varied from 20 to 100 except in one subject (Subj. 3) in whom the counts (Table III.2 and Fig. 3.6) were significantly higher (513). Since all these subjects were under medication and only one subject had a very high absolute count it is likely that he was in an acute clinical stage.

Davies et al.(1986) studied depression and anxiety in patients undergoing investigations for head and neck cancers. Significant higher depression score was observed in patients whose biopsies proved positive, than those whose biopsies were negative. Montgomery et al. (1990) examined the intermittent short lived episodes of depression and suicidal behavior. They observed that episodes occurred unpredictably with interval of 18 days in 70% episodes. This reveals the rhythmicity and the effects of solar and lunar cycles over the condition of brain for decisions.

In the present study, of the 360 patients reporting for treatment of depression (fig. 3.5) maximum number reported during new moon (NM), full moon (FM) and 5 to 6 days after FM. However, since only one lunar cycle was studied definite conclusions cannot be drawn.

6. SUMMARY

a) Whereas the quantities of all other serum protein fractions in medical students appearing for the 1st M.B.B.S. examination remained the same, both during stress i.e. before commencement of the examination as well as after the examination, there is a distinct reduction in the serum protein fraction beyond transferrin and C3 when the students are in great stress. In one student there is great reduction in protein fractions after the examination rather than before the examination.

b) Serum protein profiles of subjects of depression did not show much variation from those of normal healthy control subjects except subject no. 1 in whom a general reduction in all the serum fractions was noticed.

c) Lunar cycle studies on patients of depression reporting for treatment demonstrated that acrophases were observed on NM and 5 to 6 days after full moon. The number of patients is nearly half of the maximum number on lunar days 3 to 8.

7. REFERENCES

BERGER, B.D. and V.J.ADESSO. 1991. Gender differences in using alcohol to cope with depression. [ADDICT BEHAV. 16(5) : 315-328.]

BRANCHEY, L., U.WEINBERG, M.BRANCHEY, P.LINKOWSKY and J.MENDILEWICZ. 1982. Simultaneous study of 24 hour patterns of melatonin and cortisol secretion in depressed patients. NEUROPSYCHOBIOLOGY. 8 (5) : 225-232.

CAPLAN, R.P.. 1994. Stress, anxiety and depression in hospital consultants, general practitioners and senior health service managers. BRITISH MEDICAL JOURNAL 309 (6964) : 1261-1263.

CLARK, L.A., D.WATSON and J.LEEKA. 1989. Diurnal variation in the positive effects. MOTIV. EMOTION. 13(3) : 205 - 234.

CROES S., P.MERZ and P.NETTER. 1993. Cortisol reaction in success and failure condition in endogenous depressed patients and controls. PSYCHONEUROENDOCRINOLOGY. 18(1) : 23 - 25.

DAVIES, A.D., C.DAVIES and M.C.DELPO. 1986. (Recd. 1987) Depression and anxiety in patients undergoing diagnostic investigations for head and neck cancer. BR. J. PSYCHIATRY : 149(OCT), 491 - 493.

DUGGAN, C.F., P.SHAM, A.S. LEE and R.M. MURRAY. 1991. Can future suicidal behaviour in depressed patients be predicted? J.AFFECTIVE DISORD. 22(3) : 111 - 118.

EVANS, P., M.BRISTOW, F.HUCKLEBRIDGE, A.CLOW and F.Y.PANG. 1994. Stress, arousal, Cortisol and secretory immunoglobin A in students undergoing assessment. BRITISH JOURNAL OF CLINICAL PSYCHOLOGY. 33(4) : 575-576.

ELLICOTT, AIMEE, C.HAMMEN, M.GITLIN, G.BROWN and K.JAMISON. 1990. Life events and the course of bipolar disorder. AM. J.PSYCHIATRY. 147(9) : 1194 - 1198.

ENDERSEN, I.M., G.B. RELLING, O. TONDER, O. HYKING, B.T. WALTHER AND H. URSIN. 1991 (1992). Brief uncontrollable stress and psychological parameters influence human plasma concentration of IgM and complement component C3. BEHAV. MED. 17(4): 167 - 176.

FIRTH, J.. 1986. Levels and sources of stress in medical students. BR. MED. J. 292(6529) 1177-1180.

FRIEDMAN, J.M. KARKAVY, G.M. ASNIS, M. BOECK and J. DIFIORE. 1987. Prevalence of specific suicidal behaviours in a high school sample. AM. J. PSYCHIATRY. 144(9) : 1203 - 1206.

GALARD, R., J.M. GALLART, R.CATALAN, S.SCHWARTZ, J.M. ARGOELLO and J.M. CASTELLANOS. 1991. Salivary cortisol levels and their correlation with plasma ACTH levels in depressed patients before and after the DSI. AM. J. PSYCHIATRY. 148 (4) : 505-508 .

HEALY, D., A.O'HALLORAN, P.A. CARNEY and B.E. LEONARD. 1986. Platelet 5 hydroxytryptamine (5-HT) uptake in delusional and nondelusional depressions. J.AFFECTIVE DISORD. 109(3) : 233 - 240.

JOYCE, P.R., R.A. DONALD and P.A. ELDER. 1987. Individual differences in plasma cortisol changes during mania and depression. J.AFFECTIVE DISORD. 12(1) : 1 - 6.

KENARDY, J., L.FRIED, H.C. KRAEMER and C.B. TAYLOR. 1992. Psychological precursors of panic attacks. BR.J. PSYCHIATRY. 160 (MAY) : 668 - 673.

KUHS, H. and D.RESCHKE. 1992. Psychomotor activity in unipolar and bipolar depressive patients. PSYCHOPATHOLOGY. 25(2): 109 - 116.

LESTER, D.. 1990. Depression and suicide in college students and adolescents. PERS. INDIVID. DIFFER. 11(7) : 757 - 758.

LEWIS, L.J., K.T. BRITTON, D.L. BRAFF. 1994. Part fourteen, section 4, IN : Harrison's Principles of Internal Medicine Vol. 2 Thirteen Ed. pp 2400 - 2419.

LINKOWSKI, P., E.V.CAUTER, M.KERKHOFS, P.HUBAIN, M.BRASSEUR, R.LECLERCQ, J.GOLSTEIN, G.COPINSCHI and J.MENDLEWICZ. 1988. Neuroendochrine regualation in unipolor and bipolar depressions. NEUROPHSIOL. CLIN. 18(2) : 141 - 152.

MATUSSEK, P., O. LUKS and G. SEIBT. 1986. Partner relationships of depressives. PSYCHOPATHOLOGY. 19(3) : 143 - 156.

MONTGOMERI, S.A., D.MONTGOMERY, D.BALDWIN and M.GREEN. 1989 (1990). Intermittent 3-day depressions and suicidal behaviour. NEUROPSYCHOBIOLOGY. 22(3) : 128 - 134.

PFLUG, B., W.ENGELMANN and H.J. GAERTNER. 1982. Circadian course of body temperature and the excretion of methoxy hydroxy phenol glycol and vanillylmandelic acid in a patient with bipolar depression. J.NEURAL. TRANSM. 53 (2/3) : 213 - 216.

RAO, M.L., B.M.O. HAUSEN, A.MACKERT, B.STREBEL, R.D. STIEGLITZ and H.P. VOLZ. 1992. Blood serotonin, serum melatonin and light therapy in healthy subjects and in patients with nonseasonal depression. ACTA. PSYCHIATR. SCAND.86 (2) : 127 - 132.

ROSENTHAL, P.A., S.ROSENTHAL, M.B. DOHERTY and D.SANTORA. 1986. Suicidal thoughts and behaviours in depressed hospitalized preschoolers. AM.J.PSYCHOTHER. 40(2) : 201-202.

ROY, A., M.LINNOILA, F.KAROUM and D.PICKAR. 1986. Relative activity of metabolic pathways for norepinephrine in endogenous depression. ACTA. PSYCHIATR. SCAND. 73(6) : 624 - 628.

SCHULZ, P., C.COSTA, J.WIDMER and P.DICK. 1986. Influence of the time of administration of dexamethasone 0.25 milligrams on cortisol secretion in normal humans PSYCHOPHRAMACOLOGY. 89(3) : 293 - 295.

SMOLEN, R.C., D.A. SPIEGEL and C.J.MARTIN. 1986. Patterns of marital interaction associated with marital dissatisfaction and depression. J.BEHAV. THER. EXP. PSYCHIATRY. 17(4) : 261 - 266.

SOUETERE, E., E.SALVATI, J.BELUGOU, D.PRINGUEY, M.CANDITO, B.KREBS, J.ARDISSON and G.DARCOURT. 1988. Circadian rhythms in depression and recovery. Evidence for blunted amplitude as the main chronobiological abnormality. PSYCHIATRY.RES. 28(3): 263 - 278.

TOLOR, A. and V.M. MURPHY, 1985. Stress and depression in high school students. PSYCHOL. REP. 57(2) : 535 - 541.

TOSCA, P., L.FENOGLIO, F.ZERBI, A.ROMANI, G.BEZZI, E.FERRARI and C. CANEPARI. 1982. Neuroendocrinological aspects of depression and symptomatological picture. PSYCHIATR. CLIN. 15(3) : 153 - 15g.

VODERHOLZER, U., G.LAAKMANN, R.WITTMANN, C.D. BUJIA, A.HINZ, C.HAAG and T.BAGHAI. 1993. Profiles of spontaneous 24 hour and stimulated growth hormone secretion in male patients with endogenous depression. PSYCHIATRY RES. 47 (3) : 215 - 217.

WEISSMAN, M.M., M.FENDRICH, V.WARNER and P.WICKRAMARATNE. 1992. Incidence of psychiatric disorder in offspring at high and low risk for depression. J.AM. ACAD. CHILD ADOLESC. PSYCHIATRY. 31(4): 640 - 648.

ZOCCOLILLO, MARK, G.E. MURPHY and R.D. WETZEL. 1986. Depression among medical students. J.AFFECTIVE DISORD. 11(1): 91 -96.

E. CHAPTER 4.

Studies on Kidney Function Diseases

1. Preamble

Chronic Renal Failure is defined as reduction in glomerular filtration rate of more than 3 months duration. Irreversible reduction in glomerulofiltration rate below 5 ml/min is known as End Stage Renal Disease.

Causes : There are various causes for the development of Chronic Renal Failure.

Diabetes Mellitus, hypertension, glomerulonephritis, glomerulosclerosis, polycystic kidney disease, nephrosclerosis, pyelonephritis, other interstitial nephritis, unknown etiology, etc.

Treatment of Chronic Renal Failure

The treatment modalities are few and specific.

1) Conservative
2) Non conservative

Conservative treatment consists of dietary modification, drug therapy, identification and elimination of underlying disease, correction of numerous reversible components and prevention of further damage to the kidney.

Non conservative treatment refers to haemodialysis, peritoneal dialysis and transplantation (live related or cadaver).

Conservative Therapy

The aim of conservative treatment is to control symptoms, minimize complications, prevent long term sequelae of uremia, and slow the progression of renal insufficiency.

Dietary control plays a very important role in the management of CRF. Early restriction of sodium and fluid may be important in the treatment of hypertension. As renal insufficiency progresses, restriction of foods high in potassium and phosphates is necessary. Reduction of protein content reduces anorexia, nausea and vomiting and if initiated early may retard progression of the disease.

Every effort should be made to correct any of the numerous reversible components. e g. urethral or ureteric observation, volume depletion, etc.

Treatment of the underlying disease is most important e.g. Hypertension, UTI, Nephrolithiasis, etc.

Non Conservative Therapy - Haemodialysis consists of a process of diffusion across a semipermeable membrane to remove unwanted substances from the blood while adding desirable components. Most patients require 10-15 hours of dialysis per week.

Peritoneal dialysis is performed by placement of a catheter and a peritoneal lavage is performed hourly for 24-72 hours. The catheter is then removed.

Transplantation of the human kidney is possible today with the help of immunosuppresive drugs.

Acute Renal Failure - Acute Renal Failure is broadly defined as a rapid deterioration in renal function sufficient to result in accumulation of nitrogenous wastes in the body. The most common general cause of acute renal failure is renal ischemia.

Nephrotoxic agents viz. aminoglycoside antibiotics, radiographic contrast agents heavy metals, organic solvents, and glycols are common inducers of acute renal failure. Release of large amounts of myoglobin into the circulation is a major cause of acute renal failure.

Multiple aetiologies are likely, as in patients with shock who are volume-depleted, have received blood transfusions, are septic, and have received nephrotoxic antibiotics.

Clinical Course - The clinical course in acute tubular necrosis can be divided into an initiating phase, a maintenance phase, and a recovery phase. Infections complicate 30% to 70% of all cases of acute renal failure and are a leading cause of morbidity and mortality. The sites of infection include the respiratory tract, operative sites, and urinary tract.

Cardiovasular complications of acute renal failure involve circulatory congestion, hypertension, arrhythmias, and pericarditis.

Neurologic abnormalities are common in acute renal failure. In undialyzed patients, lethargy, somnolence, confusion, disorientation, asterixis, agitation, myoclonic muscle twitching, and generalized seizures may be observed. These neurologic abnormalities are most often encountered in the elderly patient and generally respond well to dialytic therapy.

Gastrointestinal complications of acute renal failure are common and include anorexia, nausea, vomiting, ileus and poorly defined abdominal complaints.

The recovery phase of acute renal failure commences when the glomerular filtration rate increase so that the BUN serum creatinine concentrations no longer continue to increase.

Management - The first principle of therapy in acute renal failure is to exclude causes of deterioration in renal function which are potentially remedial. A search for prerenal factors, obstructive uropathy, glomerulonephritis, renal vascular and interstitial disease, and intrarenal crystal precipitation should be performed.

General therapeutic approach to patients with acute renal failure.

1) Exclude all specifically treatable causes of decreasing renal function including correction of prerenal and postrenal factors.

2) Attempt to establish a urine output.

3) Conservative therapy .

a) Decrease intake of nitrogen, water, and electrolytes to match output.

b) Provide adequate nutrition.

c) Alter medication therapy.

d) Maintain clinical monitoring frequency of vital signs determined by patient status; intake and output, body weight inspection of wound and intravenous sites, and physical examination are required daily.

e) Maintain biochemical monitoring (frequency of BUN, creatinine, electrolytes, and blood counts will be dictated by patient status; in catabolic oliguric patients, daily determination will be needed; calcium, phosphorous, magnesium, and uric acid can be determined less often).

4) Provide dialytic therapy.

2. INTRODUCTION

Kidney function diseases are important from the viewpoint of rhythmic influence. Payne et al. (1989) observed that urinary retention was periodic in nature and increased during the newmoon than during other phases of the lunar cycle. Significant circadian rhythm has been found for urinary volume and creatinine concentration. (Howanietz et al. 1987) Sidhu et al. (1989) demonstrated circadian rhythmicity in urinary volume and excretion of creatinine calcium oxalate, uric acid and phosphate in normal subjects which was not found in idopathic stone formers.

It has been found that the increased low molecular weight proteins on aging were due to tubulopthy and this occurred at fifty and gradually progressed during aging (Narita et al. 1986).

None of the studies carried out so far have been undertaken with a view to correlate relationships between death due to kidney disease and lunar cycle.

In the present investigations, therefore, the total no of deaths due to kidney disease during a 10 year period from 1987-96 were studied in relation to the different days of the lunar cycle.

The aim of the study was to determine whether the gravitational pull of the moon and the sun played any role in augmenting / reducing the symptoms of the disease thereby causing death.

The present Chapter also embodies observation on the electrophoretic serum protein profiles of 8 subjects with Chronic Renal Failure, before and after haemodialysis, with a view to find if there are any stress induced differences in their protein profiles.

3. MATERIAL AND METHODS

a. Serum protein Profiles

Serum samples were collected from patients with chronic and acute renal failure, before and after haemodialysis. (Table IV.1) Serum proteins were analyzed by PAGE as described in Chapter 1.

b. Circalunar variations

Data on deaths due to kidney disease was collected from Nagpur Municipal Corporation, Nagpur (India 21.10 N, 12 E) during the ten year period - 1987 - 96. The total no. of deaths was plotted against the lunar days.

The distribution of deaths due to kidney disease on different lunar days during the period was split into two, five year periods (1987-1991, 1992-96) and studied. Effect of age and sexual differences on circalunar variation in deaths in human subjects was also examined. Seasonal variation in deaths due to kidney disease in human subjects was also studied during 1987-96. The data pertains to deaths occurring during hospitalization.

Table IV.1 Age, Sex, Symptoms and Treatment of patients of Chronic renal failure (1 - 6) whose serum samples were analysed by PAGE.

S.N	Age	Sex	Diagnosis	Symptoms	Treatment
1)	63	Male	DM, HT	Breathlessness nausea, vomitting oedema, feet	T.Depin R - 1 TDS CRF T.Lasix 4mg OD C.1 Alpha Vit D3 x OD T.Sarbitrate x Ods T.SandocalxBD L.Creamahin x BD
2)	44	Male	HT, CRF	oedema feet, breathlessness	T Sandocal x BD T.Lasix 40mg x OD T.Depin (R) x Tds T.Aten 50 mg x OD T.Ketasma 2 x Tds
3)	32	Male	CRF	Nil	Cap. Omez 20mg x OD Cap. Fefol 1cap x BD T.Lowphas 1xTds C.Becosules 150mg x OD Cap.Amlodepin 5mg x OD T.Aten 25mg x HS
4)	28	Female	CRF,AT	Breathlessness, vomiting	T.Depin(R) x Tds oedema feet, T.Sandocal x BD T.Lasix 40mg x OD C.1 Alpha Vit D3 x OD T.Sorbitrate x Qds
5)	65	Female	CRF	Nil	T.Sarbitrate x Qds T Sandocal x BD T.Depin (R) x Tds
6)	48	Male	CRF, HT	Breathlessness	T.Sandocal 500mg x BD T.Depin(R) 20mg x BD T.Domstal-10mg x Tds T.Raufere 150mg x HS Syp Dexorange 2tsp x 12 Hrly

4. OBSERVATIONS

(a) Protein profiles of patients of chronic and acute renal failure before and after haemodialysis - Serum samples were collected from patients with chronic renal failure or acute renal failure before and after haemodialysis. The prealbumin, albumin and transferrin bands were clearly seen after separation in 7% polyacrylamide bis acrylamide gels. It is apparent from Fig. 4.1 and 4.2 that significant changes in the serum (3µl) protein profiles do not occur in patients before and after haemodialysis. Comparison between these two serum samples of the same patient shows very little variation. In one subject only (No. 3) the post transferrin bands are faint before dialysis as compared to the serum protein profile after dialysis.

(b) Circalunar, sexual, seasonal and circannual variation in deaths due to kidney disease in human subjects - Table IV.2 and IV.3 illustrates the distribution of no. of deaths due to kidney disease in human subjects during the waxing and waning phases of lunar cycles for a ten year period from 1987-1991 of 1233 subjects and for a five year period from 1992 - 96 of 1008 subjects. The graph depicts the sum of the number of deaths on different lunar days.

The total no. of deaths on day 1, day 2, day 3 etc. of 60 waxing phases and 60 waning phases of approximately 12 lunar cycles/year for the years 1987-91 were added. Thus fig 4.3 takes into account the total no. of deaths during the first day of the waxing phase. Hence, day 16 pertains to the first day of the waning phase of 60 lunar cycles.

From the Fig. 4.3 it is apparent that acrophases occur on at Lunar day 3, 7, 10, 11, 15 (FM), 19 and 24. Nadirs are observed on lunar day 1, 6, 9, 16, 22, 27 and NM. It is obvious that there is an interval of approximately 4(± 1) days in between two consecutive peaks. This phenomenon appears throughout the graph.

Table IV.2 and fig 4.3 illustrate that the maximum no. of deaths (n = 49) occurred on full moon days and minimum on day 6 (n = 30). It is further observed that the

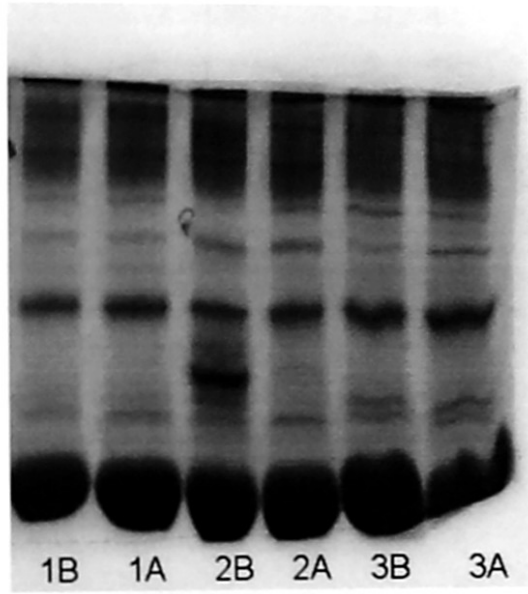


Fig.4.1 PAGE of serum (3 µL) proteins of patients of CRF / ARF : before and after haemodialysis.

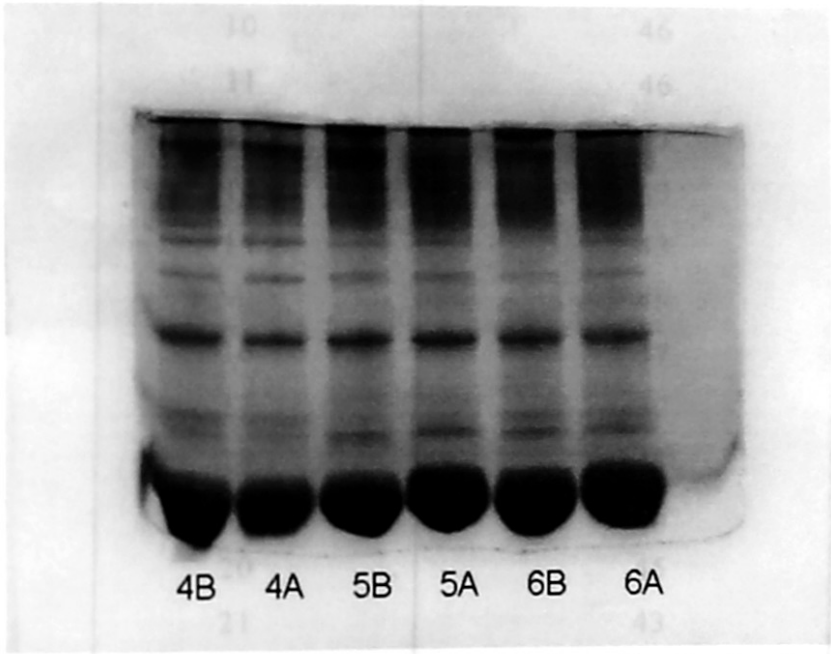


Fig. 4.2 PAGE of serum (3µL) proteins of patients of CRF / ARF : before and after haemodialysis.

Table IV.2 Circalunar Variation in Deaths due to Kidney Disease during 1987-91.

Lunar day	Number of Deaths
1	33
2	35
3	47
4	41
5	32
6	30
7	47
8	44
9	33
10	46
11	46
12	43
13	43
14	43
F.M.	49
16	37
17	37
18	43
19	46
20	45
21	43
22	42
23	48
24	49
25	44
26	39
27	36
28	37
29	36
N.M.	38

Table IV.3 Circalunar Variation in Deaths due to Kidney Disease during 1992-96.

Lunar day	Number of Deaths
1	37
2	42
3	44
4	43
5	42
6	37
7	26
8	34
9	46
10	40
11	35
12	38
13	45
14	26
FM	37
16	33
17	36
18	42
19	46
20	40
21	27
22	34
23	36
24	33
25	42
26	40
27	30
28	46
29	34
NM	28

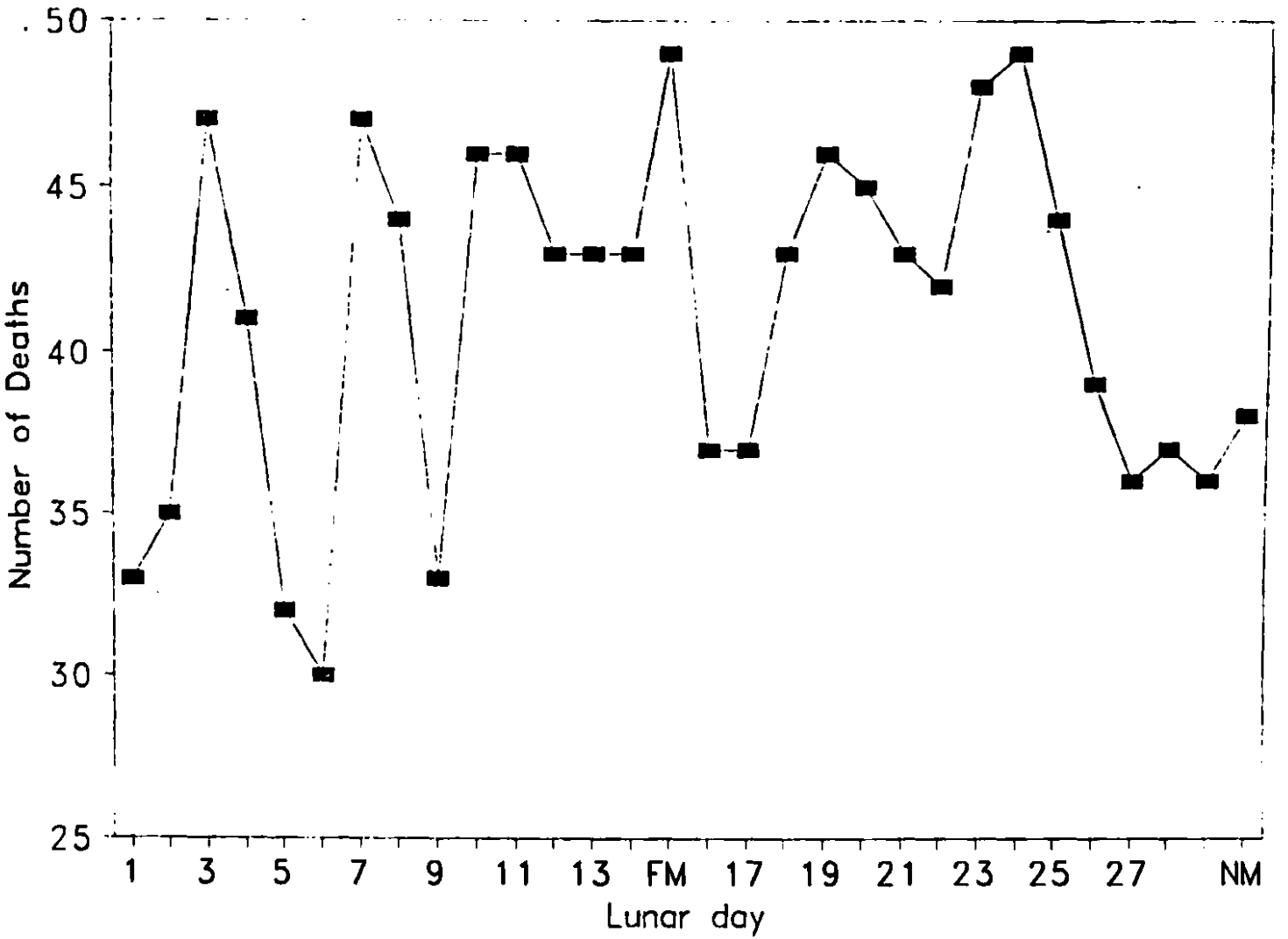


Fig. 4.3 Circalunar Variation in Deaths due to Kidney disease during 1987-91.

no. of deaths increase during the midpart of the waxing phase (n = 47 on day 7) and also during the midpart of waning phase (n = 49 on day 24).

Table IV.3 and Fig 4.4 illustrates the distribution of no. of deaths due to kidney disease in human subjects during the lunar cycles for a 5 year period from 1992 - 96 of 1008 subjects. The no of deaths on different lunar days was analyzed. The graph shows that the acrophases are on lunar days 3, 9, 13, 15, 19, 23, 25, 28. Minimum deaths are observed on lunar day 7, 11, 14, 16, 21, 27 and 30. The graph reveals that roughly there is a interval of 4 days (±.1) in between the peaks.

Table IV.2 and IV.3 and fig. 4.3 and fig. 4.4 illustrate that with the approach of the newmoon, there is a gradual decline in the number of deaths. If the total population is taken into account it is obvious that the number of deaths are on the decline with every passing year. However, it is noticed 2 days prior to and immediately after the newmoon day mortality is high. Further, it is observed that the reverse is true for full moon days. Mortality is high on full moon days with decline in numbers prior to and after full moon.

A striking feature was noticed with regard to sexual differences in mortality due to kidney disease (Table IV.4, Fig 4.5 (a) and Fig. 4.5 (b)). Of the 2241 deaths during 1987-96, 1623 were men and 618 were women. i.e. the incidence of death was 2.66 times greater in men than in women. On any given lunar day too, the chance that a man dies of kidney disease was almost twice that of a woman. Fig 4.5 (a) and 4.5 (b) illustrates that in general mortality is on the decline with every passing year. However, the decline is more with respect to men than women.

Table IV.5, IV.6 and figures 4.6, 4.7 illustrate that there is a significant effect of age on death due to kidney disease. It is noticed that after the age of 40 the number of deaths in men is three times that of women. Further, it is observed that over the years, there is a decrease in mortality in subjects below the age of 40 than those more (>) than 40 years in age.

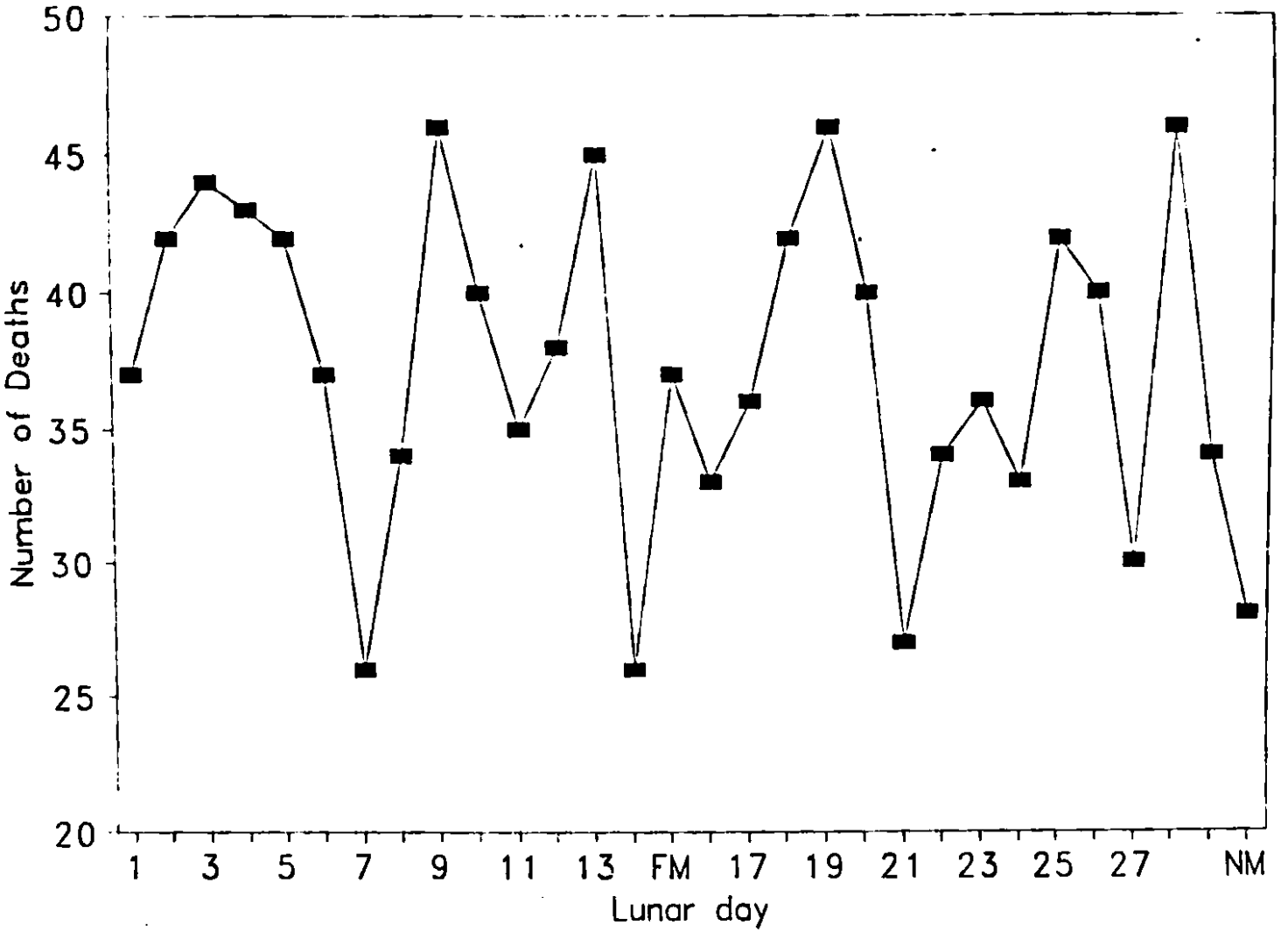


Fig. 4.4 Circalunar Variation in Deaths due to Kidney disease during 1992-96.

Table IV.4 Sexual differences in Circalunar Variation in Deaths due to Kidney Disease during 1987-96.

Year	M	F
1987	147	58
1988	208	73
1989	185	50
1990	155	78
1991	200	79
1992	161	70
1993	145	56
1994	95	47
1995	150	60
1996	177	47

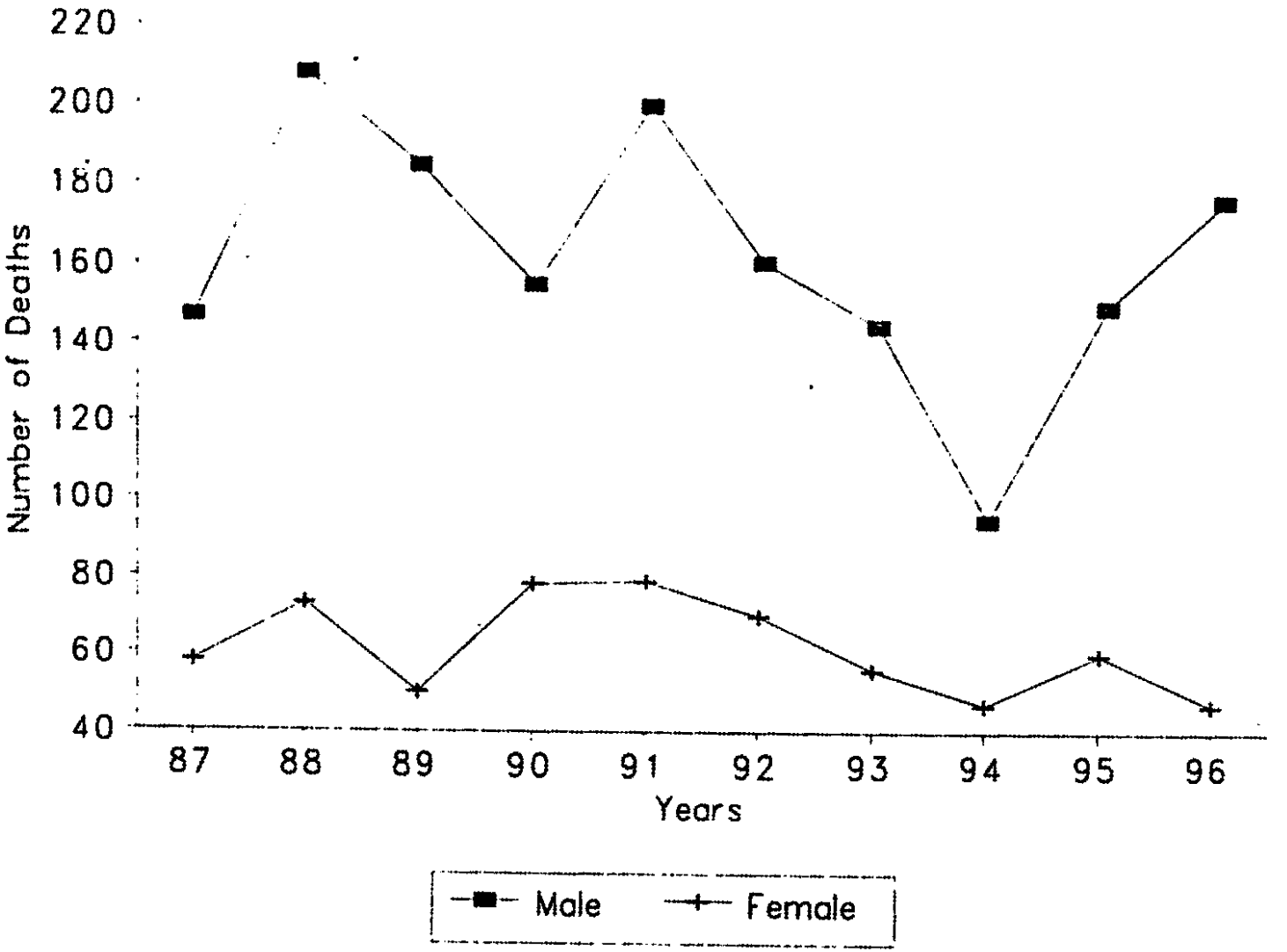


Fig. 4.5a. Sexual differences in Circalunar Variation in Deaths due to Kidney disease during 1987-96.

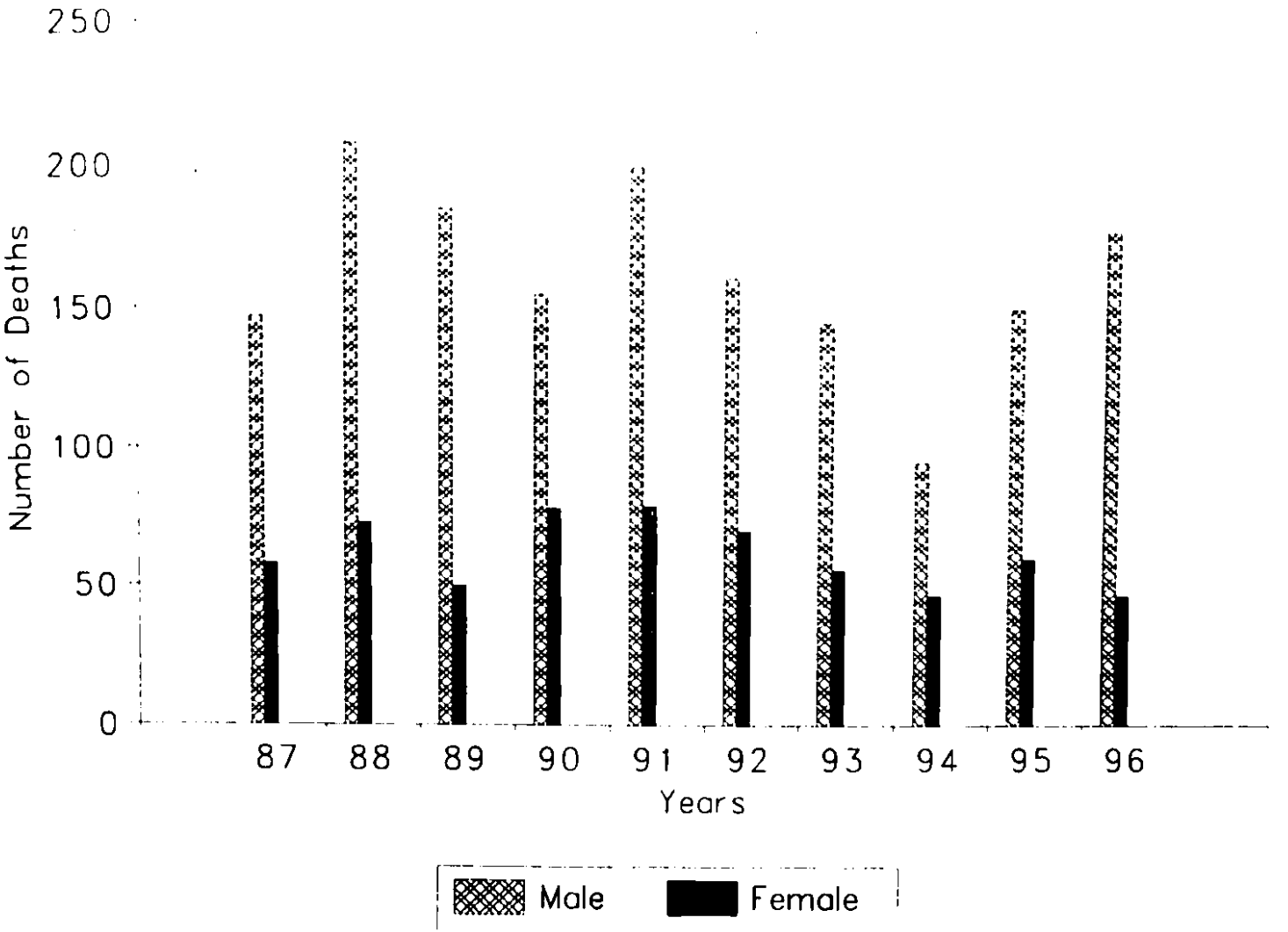


Fig. 4.5b. Sexual differences in Circalunar Variation in Deaths due to Kidney disease during 1987-96.

Table IV.5 Effect of age on Circalunar Variation in Deaths due to Kidney Disease during 1987 - 96.

Years	Less than 40	Greater than 40
1987	81	124
1988	115	163
1989	93	144
1990	78	155
1991	96	184
1992	83	148
1993	42	160
1994	33	111
1995	52	158
1996	44	180

Table IV.6 Sexual difference and effect of age on Circalunar Variation in Deaths due to Kidney Disease during 1987 - 96.

Years	Less than 40		Greater than 40	
	'M'	'F'	'M'	'F'
1987	52	29	95	29
1988	75	39	129	34
1989	68	24	117	26
1990	50	28	105	50
1991	61	35	139	44
1992	54	29	107	41
1993	29	12	116	44
1994	21	11	74	36
1995	32	20	118	40
1996	32	12	145	35

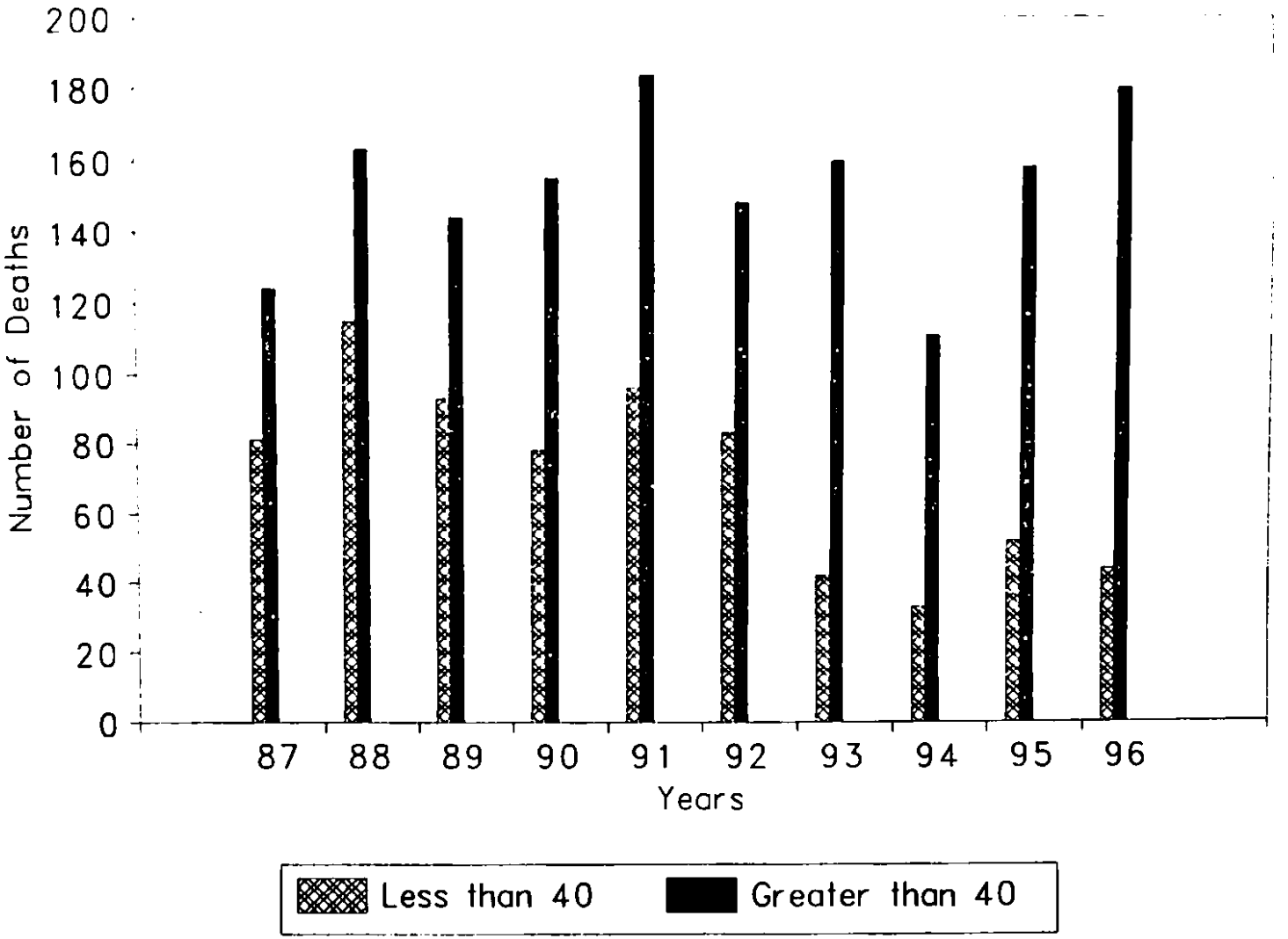


Fig. 4.6 Effect of Age on Circalunar Variation in Deaths due to Kidney disease during 1987-96.

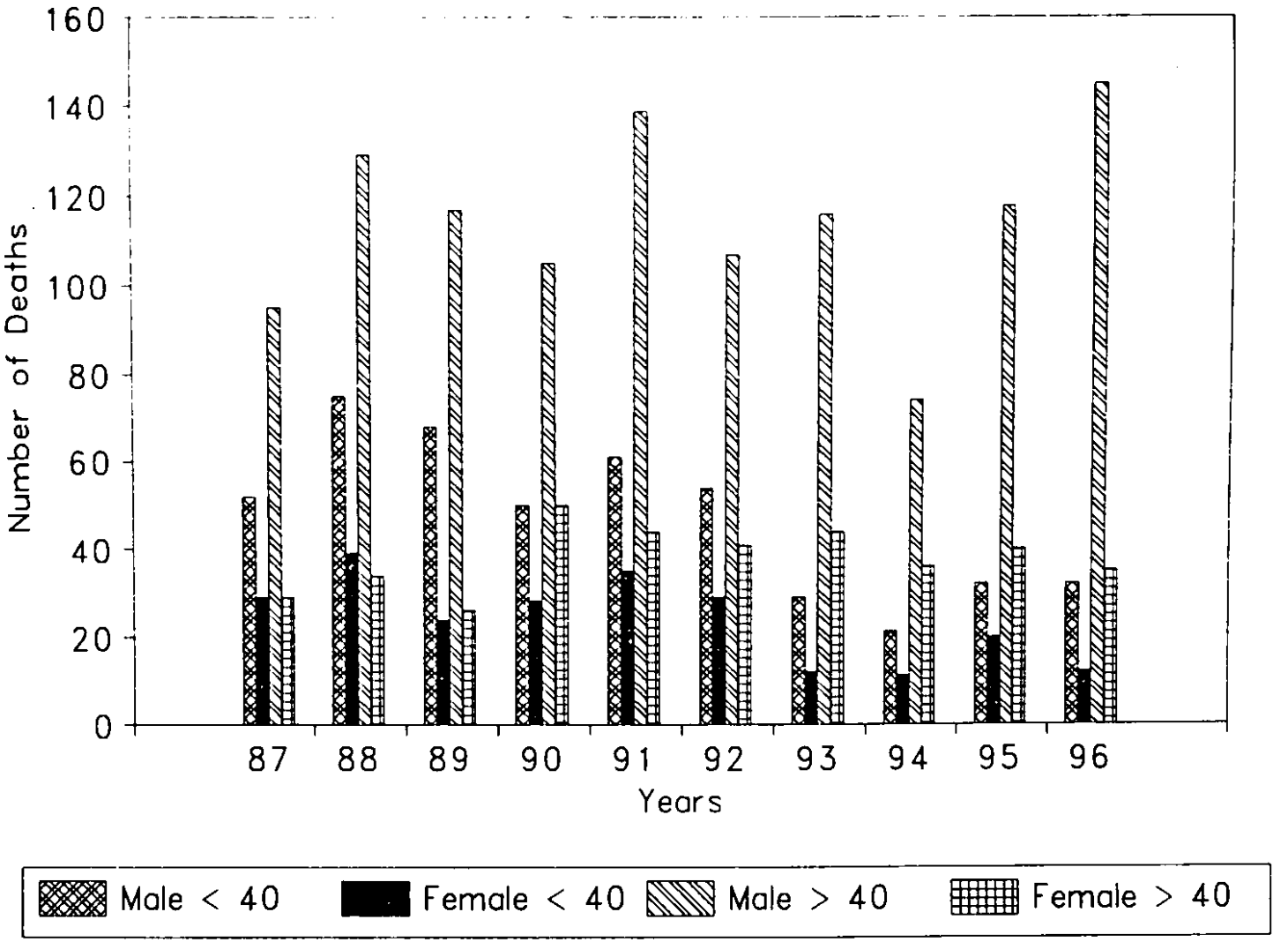


Fig. 4.7 Sexual difference and Effect of Age on Circalunar Variation in Deaths due to Kidney disease during 1987-96.

Table IV.7 and fig. 4.8 illustrates the seasonal differences in deaths due to kidney disease. In general, it was observed that the number of deaths due to kidney disease was more or less same during rainy and winter seasons and slightly less in summer season. Table IV.8, IV.9 and figs. 4.9, 4.10 illustrate, that, in general, the number of deaths during summer months (February, March, April and May) is marginally less as compared to months of rainy (June, July, August, September) and of winter (October, November, December, January) seasons.

Table IV.7 Seasonal Variation in Deaths due to Kidney Disease during 1987-96

Years	87	88	89	90	91	92	93	94	95	96
Rainy	75	90	84	83	83	92	82	50	76	82
Winter	75	102	83	90	105	84	57	51	62	67
Summer	54	95	79	70	103	62	61	43	61	75

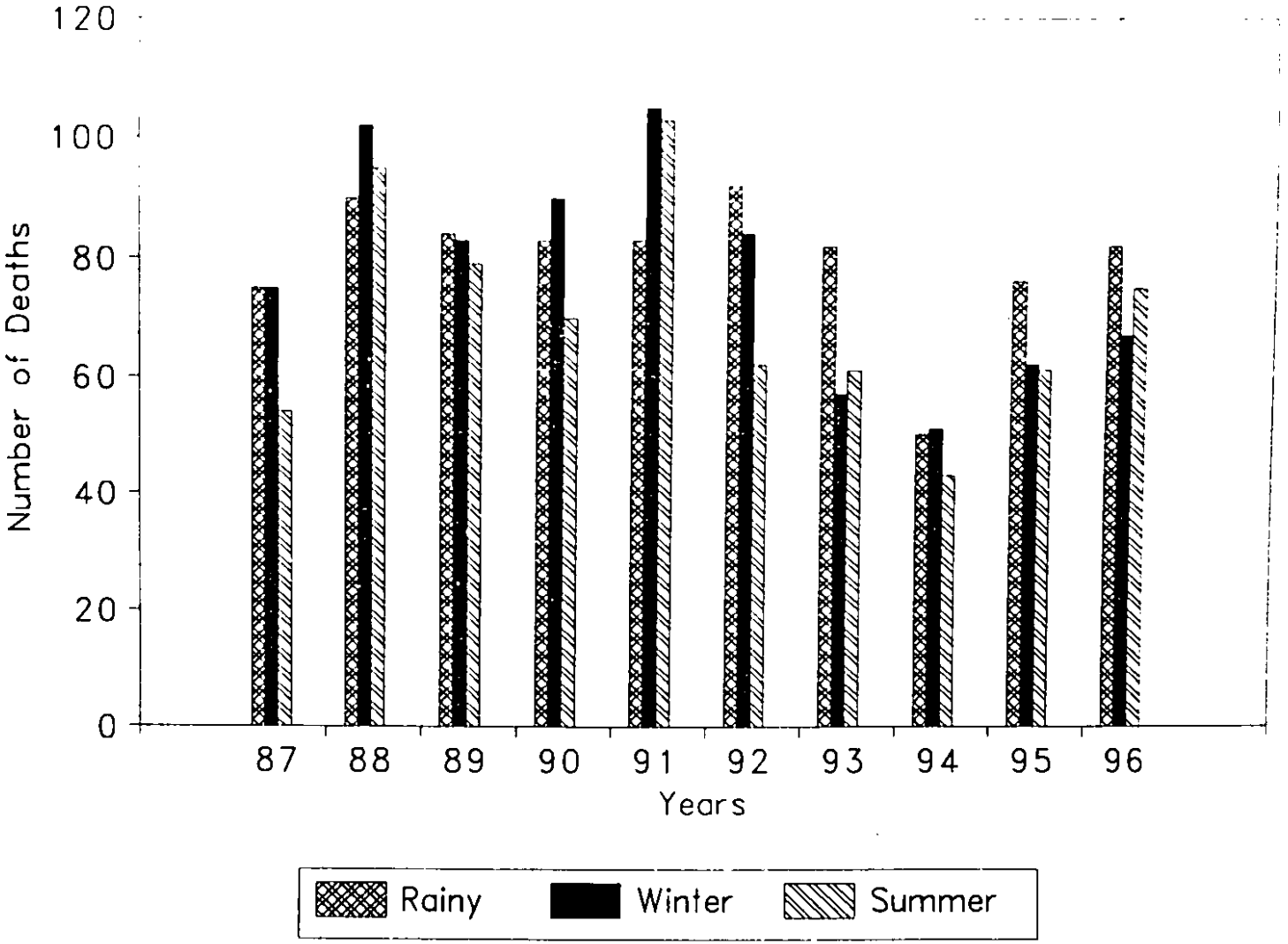


Fig. 4.8 Seasonal Variation in Deaths due to Kidney disease during 1987-96.

Table IV.8 Deaths due to Kidney Disease during 1987 - 96.

Year	Number of Deaths
1987	205
1988	281
1989	235
1990	233
1991	279
1992	231
1993	201
1994	142
1995	210
1996	224

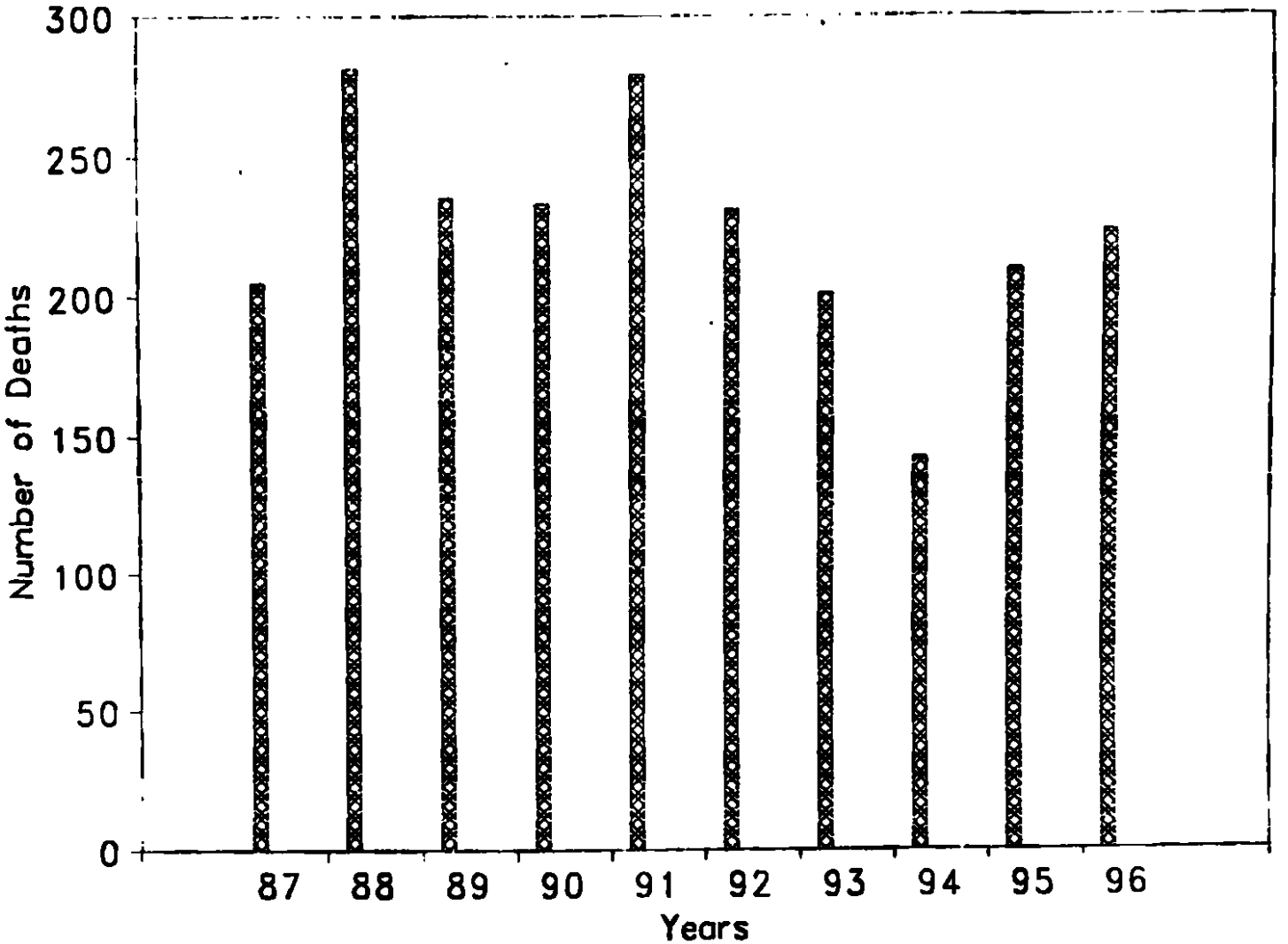


Fig. 4.9 Deaths due to Kidney disease during 1987-96.

Table IV.9 Circannual Variation in Deaths due to Kidney Disease during 1987 - 96.

Month	Number of Deaths in 10 Years
JAN	204
FEB	158
MAR	180
APR	175
MAY	189
JUN	173
JUL	194
AUG	196
SEPT	187
OCT	167
NOV	171
DEC	194

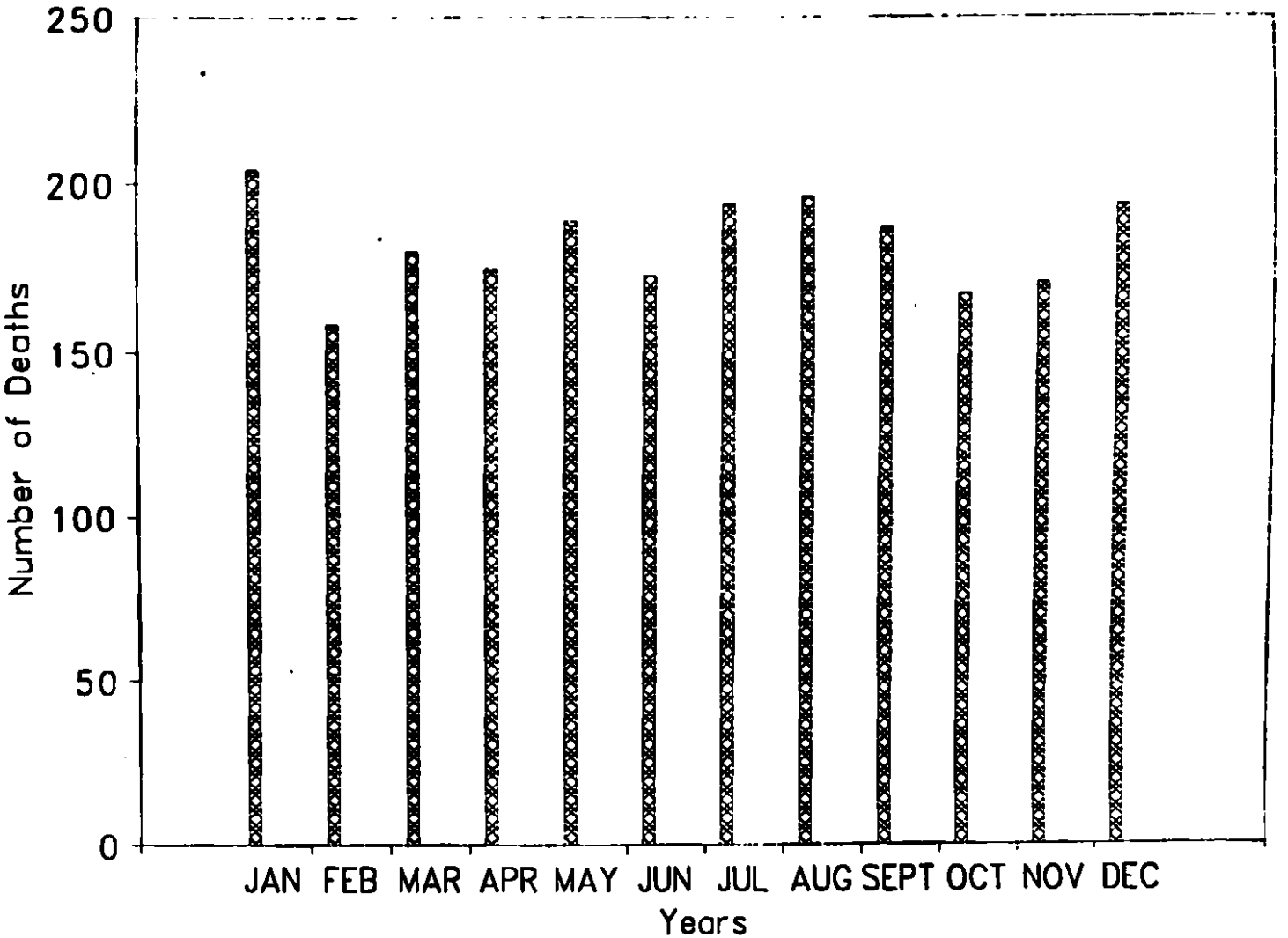


Fig. 4.10 Circannual Variation in Deaths due to Kidney disease during 1987-96.

5. DISCUSSION

Several studies have been carried out in order to determine whether any specific factors viz. electrolytes, proteins, amino acids, hormones etc. show a marked variation in kidney disease per se and before and after haemodialysis in particular. Among those that are noteworthy are mentioned below.

The circadian rhythms of sodium, K and Cl excretion in urine of patients with different stages of circulatory failure was studied by Aslanyan et al. (1990); Neorhythms were observed in patients with stage II B CF.

Brenner et al. (1994) demonstrated Na, K, Cl excretion circadian rhythm in IInd B CF. The rhythm was not present in healthy state and appeared after CF. Most probably compensatory mechanisms play a role in appearance of the new rhythms. In case of CF. like congestive cardiac failure, the cardiac output becomes less and compensates the deficit the compensatory mechanisms come into play by stimulating through the renninagiotensin aldosterone axis this may be the resource of neorhythms.

Figueirodo et al. (1996) studied fetal hemoglobin in patients with CRF. They analyzed the electrophoretic pattern of Hb in 136 patients with CRF and found increased fetal hemoglobin (HbF) levels in 7.4% of patients which contrasts with the reported 0.01 % of prevalence rate of hereditary persistence of HbF in Brazil. They noticed patients with increased HbF belonged to either continuous ambulatory peritoneal dialysis or the kidney transplantation groups. They concluded that for the HbF increase in these patients, the mechanism responsible was stress erythropoiesis.

Viljoen et al. (1992) studied the melatonin status in chronic renal failure patients. In all the CRF patients the plasma melatonin concentration was significantly increased. Successful transplantation led to reduction of melatonin levels. The circadian rhythm of melatonin secretion was suppressed in CRF patients since the nocturnal rise in melatonin was absent.

Rebeiro et al. (1996) measured plasma levels of L-arginine, Na-monomethyl-L-arginine (L-NMMA) and related amino acids in normal subjects and uremic patients before and after haemodialysis. They observed that plasma levels of L-arginine were reduced to less than half of normal values in uremic subjects when compared with controls and were not affected by haemodialysis. They also observed marked elevation of L-NMMA in uremic subjects. They suggested that uremia altered L-arginine metabolism and increased concentration of a NO synthase inhibitor was present.

Movilli et al. (1995) studied influence of protein catabolic rate on nutritional status, morbidity and mortality in elderly uremic patients on chronic haemodialysis. Elderly chronic haemodialysis (CHD) patients have reduced protein catabolic rate (PCR). Total serum proteins, serum albumin concentration, body weight and serum transferrin were determined. They recorded the incidence of hospitalization/patient/year, mortality rate and causes of death.

They concluded that CHD patients with adequate and stable Kt/V have lower PCRn values than those necessary to prevent chronic malnutrition. They also concluded that reduced protein intake did not exert any specific influence on the nutritional status morbidity and mortality and therefore age remains the strongest factor influencing mortality.

Mendes et al. (1996) studied accumulation of the endogenous L-arginine analogue Na-monomethyl-L-arginine in human end stage renal failure patients on regular haemodialysis. They measured plasma levels of L-arginine, its analogue (LNMMA) and related amino acids in normal subjects and uremic patients before and after haemodialysis. They found that plasma levels of L-arginine were reduced to less than half of normal values in uremic subjects when compared with controls and were not affected by haemodialysis. They also found markedly elevated levels of L-NMMA in uremic patients.

Paragh et al. (1993) studied lipid abnormalities in uremic patients on chronic haemodialysis. They compared cholesterol concentration and triglyceride level with

age matched control subjects and found no significant difference between the cholesterol level, but elevation in triglyceride level. They found a correlation between the lipid parameters, period spent in dialysis program and level of serum creatinine and urea.

In the present investigations it was observed that the protein profiles of patients of either acute renal failure or chronic renal failure did not vary before and after haemodialysis or from those of normal subjects.

Peuchant et al. (1994) studied lipoperoxidation (LPO) in plasma and RBC of patients undergoing haemodialysis by determining polyunsaturated fatty acids (PUFA), malondialdehyde (MDA) and hydrophobic antioxidant vit A and e. They observed significant lower levels of linoleic and arachidonic acid in plasma before dialysis as compared to healthy controls. No difference in linoleic acid, free MDA or vit E levels were observed before and after dialysis in RBC as compared to controls. They concluded that only vit A was significantly higher in haemodialysis patients and that in renal failure patients vit A offers protection from oxidative stress in erythrocytes but not in plasma.

Maltana et al. (1995) studied the effect of time of day of dialysis shift on serum biochemical parameters in patients on chronic haemodialysis. Clinical and biochemical parameters were compared. It was observed that higher potassium and phosphate levels were related to the time of day and not to age, amount of dialysis given or diet of patient. They also found that serum albumin, creatinine, sodium and chloride levels differ depending on dialysis shift. They concluded that the levels of serum predialysis biochemical parameters were mildly influenced by the time of day of the beginning of the dialysis shift.

Zainal et al. (1995) studied the pattern and outcome of 60 patients with CRF. They observed that

1) the prevalence of CRF was 24.8 patients/year.
2) 1.5 : 1.0 was the ratio of male to female patients.
3) Mean age was 51.4 ± 13.7 years.
4) Cause was unknown.

They also observed that mean blood urea and serum creatinine were 38.9 ± 9.8 mmol/L and 1.154.9 ± 458.7 µmol/L respectively. They found that majority of deaths occurred in patients who were not receiving renal replacement therapy and the mortality rate was 21.7%.

In the present studies an interesting feature was noticed with regard to sexual difference in the incidence of death due to kidney disease. The incidence of death was 2.66 times greater in men than in women (fig.4.5). On any given lunar day the chance that a man dies of kidney disease was almost twice that of a woman.

Payne et al. (1989) examined 815 patients with urinary retention. They found that there was no relation between urinary retention and circadian, monthly or seasonal rhythms. They concluded that urinary retention was periodic in nature and increased during the new moon than other phases of the lunar cycle.

Siegal et al. (1987) studied the relationship of social support to psychological adjustment in end stage renal disease patients and reported that they received considerable support from family and friends but less support from voluntary associations and leisure activities. Regression analysis indicated that psychological adjustment increased by social support.

In the present investigations the number of deaths due to kidney disease in human subjects during the waxing and waning phases of lunar cycle for two five year periods (1987 -91 and 1992 - 96) was examined. It was observed that there is an interval of approximately 4 (+/-1) days in between two consecutive acrophases. If the total population is taken into account it is obvious that the number of deaths is on the decline with every passing year considering that the population has been increasing. A significant difference was also noticed with respect to effect of age on the incidence of death. After the age of 40 the number of deaths in men is three times greater [redacted] than in women. Further, over the years, there is a decline in the number of deaths of subjects below the age of 40. With regard to seasonal differences in death due to

kidney disease it was observed that there was not much difference in the incidence of deaths during summer, winter and rainy seasons. The number of deaths were only marginally less during the summer season.

Puretic et al. (1994) studied peritoneal dialysis in children with renal failure. Dialysis treatment in children of age less than 15 years was about 5% per million in child population. In children with ARF, both haemo and peritoneal dialysis had been used equally. They observed that both children and their parents prefer haemodialysis. Children receiving chronic dialysis treatment underwent kidney transplantation. They concluded that to achieve pschological, social, family and working rehabilitation are important factors to conceive the program of peritoneal dialysis, haemodialysis and in preparing children for kidney transplantation.

Kodo et al. (1995) studied the dialysis clearance of active metabolites and the effective doses of temocapril and enalapril in haemodialysis patients. They found that enalapril showed a high clearance during dialysis and this increased as the plasma drug concentration became high. On the other hand temocapril showed lower clearance which was not dependent on the plasma drug. They concluded that temocapril can be used in haemodialysis patients.

6. SUMMARY

(a) Significant changes were not noticed in protein profiles of serum samples of patients before and after haemodialysis, except in one patient in whom there was reduction in the post-transferrin fraction.

(b) Circalunar variation in deaths due to kidney disease during 1986 to 1996 indicates that there is an interval of approximately 4 days between consecutive acrophases.

(c) There is a steady decline in number of deaths due to kidney disease over the years particularly among men.

(d) After the age of 40 the number of deaths due to kidney disease is approximately 2.6 times more in men than in women.

7. REFERENCES

ASLANYAN, N., L., S.L. EOLYAN, K.L.DANIELYAN and R.D. PARSYAN. 1990. Changes in the circadian rhythms of sodium, potassium and chlorine excretion with urine of patients with circulatory failure. TER. ARCH. 62(11) : 73-76.

BRENNER, B.M., J.M. LAZARUS. 1994. Part Nine, Chapter 236, Acute Renal Failure In: Harrison's Priciples of Internal Medicine. Vol. 2 Thirteenth Ed. pp. 1265 - 1273.

FIGUEIREDO, M.S., E.Y.S.KIMURA, J.O.BORDIN and J.KERBABY. 1996. Fetal haemoglobin in patients with chronic renal disease. BRAZILIAN JOURNAL OF MEDICAL AND BIOLOGICAL RESEARCH 29(8) : 1001-1004.

HOWANIETZ, H.S. KIRCHER, E.SCHOBER, W.MARKTL, N.KLAMMER, M.GRUSKA and G.LUBEC. 1987. Chronobiology of urinary acid glycosaminoglycan excretion. PAEDIATRPAEDOL. 22(1) : 13 -18.

HUGH, R.B., and B.M. BRENNER. 1994. Part Nine, Chapter 237, Chronic Renal Failure In: Harrison's Priciples of Internal Medicine. Vol. 2 Thirteenth Ed. pp. 1274 - 1280.

KODO, Y., H.SAITO, K.SASAHARA and Y.HIRASAWA. 1995. Clearance of temocapril and enalapril during haemodialysis treatment. Clinical Drug Investigation 9(4): 232-238.

MALTANA, J., A.PATEL, J.D.WAGNER, J.K.MAESAKA and P.C.SINGHAL. 1995. Effect of time of day of Dialysis shift on serum Biochemical parameters in patients on chronic Haemodialysis. American Journal of Nephrology 15(3) : 208-216.

MENDES, R., A.C., N.B. ROBERTS, C.LANE, M.YAQOOB and J.C.ELLORY. Accumulation of the endogenous L-arginine analogue N^G-Monomethyl-L-arginine in hu-

man end stage renal failure patients on regular haemodialysis. EXPERIMENTAL PHYSIOLOGY 81(3) : 475-481.

MOVILLI, E., M.FILLIPPINI, G.BRUNORI, M.SANDRINI, E.COSTANTINO, L.CRISTINELLI and R.MAIORCA. 1995. Influence of protein catabolic rate on nutritional status, morbidity and mortality in elderly uremic patients on chronic haemodialysis. NEPHROLOGY DIALYSIS TRANSPLANTATION. 10(4): 514-518.

NARITA, M., A.KOYAMA, H.SUKURAI, Y.SUDOU, H.INAGE, T.KOKO and S.TOJO. 1986. Effect of aging on the construction of urinary proteins in normal adults. PHYS. CHEM. BIOL. 30(4) : 257 - 264.

PARAGH, G., Z.BALOGH, J.MATYUS, I.KARPATI, L.VJHELYI, G.KAKUK and A.LEOVEY. 1992-1993. Lipid abnormalities in uremic patients on chronic haemodialysis. ACTA MEDICA HUNGARICA 49(3-4) : 207-217.

PAYNE, S.R. , D.J.DEARDON, G.F. ABERCROMBLE and G.L.CARLSON, 1989. Urinary retention and the lunisolar cycle. BR. MED. J. 299 (6715) : 1560 - 1562.

PEUCHANT, E., M.A.CARBONNEAU, L.DUBOURG, J.THOMAS, A.PERROMAT, C.VALLOT and M.CLERC. 1994. Lipoperoxidation in plasma and RBC of patients undergoing haemodialysis: vit A, E and Iron Status. Free radical Biology and Medicine 16(3): 339-346.

PURETIC, ZVONIMIR, Z.MAREKOVIC, L.B. FILIPI, S.G.BORAS, J.SLAVICEK and J.PASINI. 1994. Peritoneal dialysis in children with renal failure. ACTA FACULTATIS MEDICAE FLUMINENSIS. 19(1) :13-19.

SIEGAL, B.R., R.J. CALSYN and R.M. CUDDIHEE. 1987. The relationship of social support to psychological adjustment in end-stage renal disease patients. J.CHRONIC DIS.. 40(4) 337 - 344.

SIDHU, H., S.VIDYANATHAN, D. WANGOO, S.K. THIND, R.NATH, G.C. MALA - KONDAIAH and K.KRISHNAN. 1989. The loss of circadian rhythmicity in urinary solute excretion in idiopathic stone formers. BR. JUROL. 64(4) : 333-335.

SIDHU, H., S.K. THIND, R.ANTH, S. VIDYANATHAN A.K. HEMAL, A.K. MANDL, and K.KRISHNAN. 1989. Comparative study of the circadian rhythmicity in the urinary concentration of glycosaminoglycans in patients of calcium oxalate nephrolithiasis and in healthy adults. UROL. INT. 44(4); 218 - 221.

VILJDEN, M., M.E.STEYN, B.W.J. VAN RENSBURG and S.G. REINACH. 1992. Melatonin in chronic renal failure. NEPHRON. 60(2) : 138-143.

ZAINAL, D., M.MONNIATY and N.NAZIMI. 1995. The pattern of CRF in Kelantan, Northeastern state of Malaysia. Southeast Asian Journal of Tropical Medicine and Public Health. 26(4) : 781-784.

F. CONCLUSIONS

a) Neither the serum protein profiles (as studied by polyaryalamide disc gel electrophoresis) nor the immune status of mentally retarded children (as refelected by the absolute count of CD_3 positive T cells and CD_{19} positive B cells) was at variance from those of age matched control children thereby indicating that mental retardation per se is independent of these two factors. Since mentally retarded children were perceived as having negligible stress, the above findings are in agreement with the anticipated results.

b) As the levels of all electrolytes studied, particularly Ca, Mg, Fe and Mn is strikingly high in mentally retarded children it is likely that mental retardation may partly be due to high concentration of these electrolytes in the diet during childhood or due to genetic/physiological factors which cause accumulation of these electrolytes in body fluids. The above view has support from the observation that the electrolyte concentrations in one normal child, (who had a sibling who was mentally retarded) were between those of mentally retarded children and those of normal children.

c) Acute stress caused by labour pains induces distinct reduction in the 7S globulin fraction of serum proteins. The levels of these proteins are restored after delivery.

d) Postoperative trauma too causes a reduction in specific protein fractions (post transferrin) after delivery by caesarean section. These findings clearly point out that these proteins have a distinct role to play during stress caused by acute pain.

e) The remarkable reduction in the same serum protein fractions as observed in other stress situations, viz. in students just prior to commencement of a crucial examination (1st M.B.B.S) and restoration of the said protein band in polyacrylamide gels after completion of examinations clearly indicates that acute stress, irrespective of the cause, evokes a similar response.

f) Since neither the protein profiles nor the absolute counts of CD_{57} positive Natural killer cells showed variation from those of age-matched control subjects, it may be concluded that the continuously depressed state does not evoke any change in the 7S globulin fraction of serum

proteins. An exception to the above was the case of one depressed subject whose absolute CD_{57} positive Natural Killer Cells count was extremely high indicating probably that he was in a truly acute clinically depressed state since Siedel et al. (1996) has also reported high CD_{56} positive lymphocyte counts in acute states of depression.

g) Although patients of acute/chronic renal failure were perceived to be in great stress, in situations when they underwent dialysis, it was found that there was no change in any of the serum protein fractions either before or after dialysis. Considering that 3 of these patients (subjects no.2, 4, 6) died within a day or two after serum samples were analysed, it may be concluded that stress, if any, that is induced by dialysis need not be reckoned with and factors other than stress come into play at the time of death.

h) Several interesting conclusions could be drawn from a study of the effect of lunar cycle, age, sex, seasonal variation and annual variation in deaths due to kidney disease. With respect to lunar cycle variation, there appears to be a 4-5 day interval between successive acrophases with peaks occuring on full moon days and very few deaths on new moon days, thereby indicating that gravitational forces of the sun, moon and earth, all have a role to play. Since awareness and facilities for medical treatment have increased considerably over the years, the number of deaths that occur annually has remained almost the same (200-250) over the last 10 year period, although the population has increased enormously. Further, the incidence of deaths due to acute/chronic renal failure in men is more than twice that of women and this is particularly true in subjects above the age of 40. No seasonal variations were observed in the incidence of deaths thereby indicating that change in season has no effect on the incidence of deaths.

i) The amino acid sequence of the protein/s that are depleted during varied stress situations, the source of the mRNA that codes for the proetin/s, the base sequence of the coding genes, and whether regulation is at the transcriptional or translational level will be worth investigating.

ACKNOWLEDGEMENTS

I express my deep sense of gratitude to Dr. (Mrs.) S.A.Bhide, M.Sc., Ph.D., Reader in Zoology, Institute of Science, Nagpur for her inspiring guidance, keen interest and active support throughout the course of these investigations.

My thanks are due to Prof. S.A. Suryawanshi, M.Sc., Ph.D., Director, Institute of Science, Nagpur for providing me all the necessary facilities and constant encouragement for carrying out this work.

I am equally thankful to Dr. M.S. Oolaniya, Dr. (Mrs.) A.D.Ookalkar, Dr. D.S.Ookalkar, Nephrologist, Dr. A.D. Joshi, Psychiatrist, E.S.I.S. Hospital, Nagpur, Dr. (Mrs.) P. Risbud, Gynecologist Matru Seva Sangh Hospital, Nagpur for granting me permission to collect the data and blood samples from their hospitals during the course of this work.

It is a pleasure to express my sincere thanks to all the staff-nurses and workers of the Matru Sewa Sangh, Nagpur, Ashwini Nursing Home, Nagpur, and E.S.I.S. Hospital for their kind co-operation.

I also offer my sincere gratitude to Mr. A.D. Bhide, and Mr. Manish A. Bhide for their kind co-operation.

I acknowledge with thanks the financial assistance granted by the Government of Maharashtra for awarding me the Research fellowship for three years during the course of these investigations.

S.Puranik

Nagpur :

Date : 5.7.97

Mrs.Shubhangi S.Puranik
Departmentof Zoology,
Institute of Science, Nagpur.