CD4+ AND CD8+ T-LYMPHOCYTE COUNTS AND SERUM LEVELS OF IgG IgA AND IgM IN PATIENTS WITH END STAGE RENAL DISEASE ON HAEMODIALYSIS AND CHRONIC RENAL FAILURE NOT ON HAEMODIALYSIS.

A DISSERTATION PRESENTED IN PART FULFILLMENT FOR THE DEGREE OF MASTER OF MEDICINE (INTERNAL MEDICINE) OF THE UNIVERSITY OF NAIROBI.

BY

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DECLARATION

I declare that this dissertation is my own original work and has not been published elsewhere or presented for a degree in any other University.

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DEDICATION

To my beloved wife Lilian and daughter Caroline for their support during the study.

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LIST OF ABBREVIATION

CD4+ T-lymphocytes	-	T- Helper cells
CD8+ T-lymphocytes	-	T- Suppressor cells
CRF	-	Chronic Renal Failure
ELISA	-	Enzyme Linked Immunosorbent Assay
ESRD	-	End Stage Renal Disease
Hb	-	Haemoglobin
HIV	-	Human Immunodeficiency Virus
Ig	-	Immunoglobulin
IL	-	Interleukin
KEMRI	-	Kenya Medical Research Institute
K.N.H	-	Kenyatta National Hospital
RBC	-	Red Blood Cell
WBC	-	White Blood Cell

SUMMARY

This study was conducted at the Kenyatta National Hospital Renal Unit, renal clinic and medical wards to investigate some immunological parameters in non-haemodialysing CRF patients and ESRD on haemodialysis. The parameters studied included CD4+ and CD8+ T-lymphocyte counts and serum concentration of IgG, IgA and IgM.

The objective of the study was to find out whether there is a significant difference in the immunological status at the different stages of renal failure as compared to the normal controls.

A total of 90 individuals were investigated, comprising of 30 patients with ESRD on haemodialysis, 30 non-haemodialysing CRF and 30 normal controls. Patients diagnosed to have CRF or ESRD who were currently on haemodialysis and normal controls who fulfilled the inclusion criteria were recruited into the study. The individuals investigated were aged between 12 and 60 years, lacked clinical evidence of infection, disease or drug use known to interfere with the immune system. Blood samples were taken (before haemodialysis in cases of ESRD) and the immunological parameters analyzed. A non-parametric data analysis using SPSS computer package was done.

The results revealed aberrant counts of total lymphocytes, CD4+ and CD8+ T-lymphocyte in both non-haemodialysing CRF and ESRD patients on haemodialysis compared to the normal control. These included low lymphocyte count with normal total WBC count, low absolute CD4+ and CD8+ T-lymphocyte count. Serum concentration of IgG, IgA and IgM were normal in the uraemic patients compared to the control group. High CD4+ : CD8+ T-lymphocyte ratio was observed in ESRD patients on haemodialysis as compared to that in non-haemodialysing CRF.

It is concluded that non-haemodialysing CRF and ESRD on haemodialysis have reduced lymphocyte count, absolute CD4+ and CD8+ T-lymphocyte count. They both have normal concentration of IgG, IgA and IgM compared to the control group. Higher CD4+: CD8+ T-lymphocyte ratio is observed in ESRD patients on haemodialysis.

INTRODUCTION AND LITERATURE REVIEW

i) General overview of Immune status in Uraemia

Immunodeficiency is a well established finding in uraemic patients (1,2). This is reflected by the increased susceptibility to infections, increased incidence of cancer, burn-out of immunologic disorders and inadequate response to *antigenic challenge e.g. against hepatitis B-vaccine and BCG.*

Impaired immune response in uraemia may be caused by multiple derangements of the immune system (3). These include, malnutrition, inefficiency of mucocutaneous barriers, granulocyte abnormalities (locomotion and phagocytosis), changes in immunoglobulin levels and synthesis, reticuloendothelial function and cell mediated immunity (4).

Cellular immunity primarily involves T-Lymphocytes and the lympokines they produce. There is an inter-relation with granulocytes and macrophages as effector cells of this system. Although the number of circulating phagocytes is not affected, granulocytic and macrophagic differentiation may be altered (5). Specific phagocytic functions, such as microparticle ingestion and killing of bacteria have been studied extensively, with contradictory results, the majority of which point to suppression (4). Suppression of cell mediated immunity in uraemia probably involves intrinsically normal

cells in a primary immunosuppressive milieu. There is a substantial number of cellular dysfunctions (6) including T-Cell growth factor activity, T-cell subset identification, metabolic responsiveness, lymphatic immune response and/or proliferation and E-rosette forming capacity. It has been demonstrated that B-cell activation and immunoregulation, as well as helper T-cell functions are quantitatively deficient (7). A defective IL-2 production (8) and the retention of guanidine compounds (9) have been recognized as potential causative factors.

Despite a considerable number of reports, there still remains an incomplete understanding of both the clinical and biochemical characteristics of this immune deficiency (10).

ii) <u>Lymphocyte response in uraemia</u>

A quantitative defect in immuno-competent cells is not by itself responsible for the immune deficit observed in uraemia. Several studies have shown that in-vitro blastogenic response of uraemic lymphocytes to various antigens including alloantigens is significantly lower than that of normal controls (11-14). Although several hypothesis for this unresponsiveness has been put forward, its basis is still controversial. For several years, it was suggested that the defect was not intrinsic to the lymphocyte but depended on the

presence in uraemic serum of soluble factors having a suppressor effect (12, 15, 16). This inhibitory capacity of uraemic sera was described as having both dialyzable and non-dialyzable components. The dialyzable components include urea and creatinine which are non-toxic to lymphocytes, and methyl-guanidine which severely impair lymphocyte blastogenesis at concentrations similar to those found in uraemic serum (17). However, despite much effort, the suppressor substance(s) have not been precisely characterized, at least in man.

Apart from the underlying mechanism, most recent findings have confirmed that the defective proliferation response of uraemic lymphocyte correlates with an abnormally low bio-availability of IL-2 (8, 13, 14, 18).

IL-2 which represents a major T-cell derived growth factor, and is essential for T-lymphocyte proliferation and differentiation is almost undetectable in supernatant from stimulated T-cell cultures from both non-haemodialysed and haemodialysed uraemic patients (18). Patients' T-cells may regain a normal proliferative capacity when cultures are supplemented with purified exogenous IL-2 (14).

Production of IL-2 by T-cells is regulated by monocytes and macrophages. These cells produce IL-1 required for both IL-2 production and IL-2 receptor expression. A recent report

suggests that the low IL-2 levels could be attributed to reduced function of uraemic monocyte (14). Analysis of IL-2 consumption relies on the study of its natural ligand i.e. IL-2 receptor on its membrane - associated or soluble form.

Under physiological conditions, the IL-2 receptor is expressed only at the surface of activated T-lymphocytes (19) and not on resting T-cells. Abnormally high proportions of circulating T-cells expressing high affinity IL-2 receptors have been found in haemodialysis patients (13, 18). This increase in both cell associated and soluble IL-2 receptor may lead to increased IL-2 consumption through absorption, thus explaining the decreased lymphokine bio availability (8,13,18).

iii) <u>T-cell subset in haemodialysis patients</u>

In patients undergoing haemodialysis, profound lymphopenia as well as altered proportion and decreased numbers of CD4+ and CD8+ T-lymphocytes subsets exist (10).

CD4+ T-lymphocyte comprise the T-helper functions subset important for the initiation of immune response and crucial for immune reaction. Naturally, the decreased absolute number of CD4+ T-lymphocytes in CRF result in altered or ineffective function in immune regulation and is probably an important factor in the immunodeficiency in uraemia (20). It is also a possible explanation for the high susceptibility to fungal, bacterial and viral infections, as well as neoplasms (21).

Previous reports (20-23) revealed reduced absolute numbers of the helper/inducer subset in uraemic patients. The proportion of CD4+ T-lymphocyte compared to CD8+ T-lymphocyte are mostly reported to be low (21,24,25), but some studies report it to be unchanged (13).

CD8+ T-lymphocytes are more diverse in function and have either suppressor activity crucial for regulation of the immune response or cytotoxic activity known to be important in tumor immunity and in viral infection. The proportion of CD8+ compared to CD4+ T-lymphocytes in haemodialysis patients has not been shown to be different from the normal controls (10, 13, 21, 26, 27). Decreased absolute values of CD8+ T-lymphocytes has uniformly been noted (3, 10, 25, 27). The CD4+:CD8+ T-lymphoctes ratio in groups of haemodialysis patients was found to be comparable to that of normal controls (3, 13, 26). Reports of increased (28) or decreased (21, 24) value of this parameter in uraemia exist.

iv) <u>B-cell response in uraemia</u>

Concerning the cell involved in humoral axis of immunity, great interest has been focused on determining whether B-lymphocyte activation and/or B-cell derived molecules contribute to the acquired immunodeficiency in patients with ESRD (22). Previous quantitative studies (20,28) have shown evidence of low number of B-lymphocytes in haemodialysis

patients. The low number of B-cells could partly account for the altered antibody response in uraemia.

Specific in-vivo and in-vitro antibody responses are significantly impaired in uraemic patients (29). However, it is not clear whether the defect is intrinsic to B-lymphocytes or linked to a defect in T-B cell cooperation, since T-cells produce several lympokines that are fundamental for B-cell differentiation (13,29). In vivo studies show low specific antibody titres during vaccination which is thought to be linked to a defect in cooperation between helper T-cells and B-cells rather than to aberrant B-lymphocyte function (8,13,29). In-vitro B-cell from haemodialysed patients produce low amounts of IgG following T-cell dependent stimulation (29). In the absence of stimulation, uraemic B-lymphocytes produce abnormally high IgM and IgG titres (E. Rothschild and L.Chatenoud, unpublished data). Thus, uraemic patients like some patients with autoimmune diseases show polyclonal B-cell activation in basal conditions but are incapable of mounting an adequate antibody response following stimulation. No sign of B-cell activation has been observed in haemodialysed patients (13).

JUSTIFICATION OF THE STUDY

Despite a considerable number of reports, there still remains an incomplete understanding of both clinical and biological characteristics of the immune deficiency that occurs in non-haemodialysing CRF and ESRD on haemodialysis. It is for this reason that further research is still necessary. There is also need for local data and to check for any difference with available Western data.

HYPOTHESIS

There is no significant difference in immunological parameters in non-haemodialysing CRF patients, ESRD on haemodialysis and normal controls.

OBJECTIVES

Broad Objective:

To analyse quantitative changes in immunological parameters in non-haemodialysing CRF patients and ESRD on haemodialysis as compared to the normal controls.

Specific Objective:

i) To determine absolute and relative CD4+ and CD8+ T-lymphocyte counts in non-haemodialysing CRF patients, ESRD on haemodialysis and in normal controls.

ii) To measure serum IgG, IgA and IgM concentration in non-haemodialying CRF patients, ESRD on haemodialysis and in normal controls.
(IgD and IgE were not assayed due to their very low levels in the serum and also due to financial constrain and time limit).

ETHICAL CONSIDERATION

Study approval by the Kenyatta National Hospital Ethical and Research Committee was sought and granted prior to commencing the study.

An informed written consent was sought from the patients and the normal control group. They were told precisely what the study was all about and that blood samples would be taken for analysis. They were also told clearly that they were under no obligation to participate in the study, and that decline to participate in the study would not affect their treatment in any way whatsoever.

MATERIALS AND METHODS

Study Design

The study population consisted of three groups: non-haemodialysing CRF patients, ESRD on haemodialysis and Interested healthy blood donors as controls. CD4+, CD8+ T-lymphocyte count, and serum concentration of IgG, IgA and IgM were analysed and compared with the control Individuals.

Study setting

The study was conducted at the Kenyatta National Hospital Medical wards, renal unit and blood donor centre (No.10). Patients with ESRD on haemodialysis were recruited from the renal unit. Non-haemodialysing CRF patients were recruited from the medical wards and normal control individuals were recruited from the blood donor centre.

ESRD patients were on regular haemodialysis treatment that was carried out for 10-15 hours in a week in 2-3 sessions. All the study patients were in clinically stable state of health at the time of recruitment. They didn't have any clinical evidence of infection or disease process known to interfere with immune system. In addition, they were not taking any medication or drugs known to interfere with immunity (see clinical evaluation below).

ESRD patients on haemodialysis and non-haemodialysing CRF patients already had random blood sugar and HIV ELISA test

done on them during their workup for the renal failure. The normal control individuals were precounselled for the HIV ELISA test by the hospital counsellors before donating blood.

The individuals studied were aged between 13 and 60 years. The patients were matched for age and sex with the normal control. The age of control individuals were matched by plus or minus 2 years to the study patients. An informed consent was then signed.

Clinical evaluation

History was taken to find out whether the individual were taking medication known to interfere with immune system. This included steroids, cytototics, antithyroid drugs, non steroidal anti inflammatory drugs (NSAIDS), cigarette smoking and alcohol. Medical conditions associated with immune deficiencies were also sought. This included diabetes mellitus, leukemia and other malignacies, malnutrition and primary immune deficiencies.

Physical examination was thereafter done to rule out clinical evidence of infection. Study cases were to have normal physical examination with no history of medical condition suggestive of possible interference with immune system.

Blood samples were then taken for investigation. HIV ELISA positive cases and individuals with haemogram picture suggestive of infection were excluded from the study.

A total of 10ml of venous blood was taken from the control individuals. This was for haemogram (2ml), serum creatinine (3ml) andHIV ELISA (2ml). At the same time 3ml of blood was taken from the matched patients for the lymphocyte differential count and serum immunoglobulins (see laboratory investigations below). Blood samples were taken between 9.00Am and 11.00Am due to low ACTH levels at this time as per the diurnal rhythm (high ACTH levels may interfere with lymphocyte count). Five HIV ELISA positive control individuals were excluded from the study. Also excluded were four individuals with haemogram picture suggestive of infection. Random blood sugar was not done on our normal control individuals due to financial contraints, however none of them had symptoms and signs suggestive of diabetes mellitus.

Sample size estimation (30)

$$N=\frac{\left\{Z\alpha\sqrt{P(1-P)\left(\frac{1}{q_1}+\frac{1}{q_2}\right)}+Z\beta\sqrt{P_1(1-P_1)\left(\frac{1}{q_1}\right)+P_2(1-P_2)\left(\frac{1}{q_2}\right)}\right\}^2}{(P_1-P_2)^2}$$

where:

$Z\alpha$ = 1.96 when α = 0.05.

$Z\beta$ = 1.282 when β = 0.10.

q_1 = proportion of subjects in CRF ; 50%.

q_2 = proportion of subjects in controls ; 50%.

N = total number of subjects required.

P_1 = proportion of CRF patients noted to be immunosuppressed ; 60%.

P_2 = Estimated immunosuppressed normal controls ; 5%

$P = q_1P_1 + q_2P_2$

Formula application for the proposed study, past study by Montgomeric et al showed that 60% of patients with CRF had immunological disorders (31).

On formula substitution, N = 27. Approximate sample size was therefore 27 for each group at 5% level of significance with a precision of 10%.

In this study, a total sample size of 90 was taken and comprised of 30 normal controls, 30 non-haemodialysing CRF and 30 ESRD patients on haemodialysis.

Case sampling

This was done by recruiting every second non-haemodialysing CRF patient satisfying the inclusion criteria as they were diagnosed in the various wards and clinic during the study period. All patients with ESRD undergoing haemodialysis who satisfied the inclusion criteria were included in the study.

Definitions of the study groups:

i) **Control:**

- Healthy individuals matched for age and sex to non-haemodialysing CRF and ESRD patients on haemodialysis.
- Normal renal function with no immunological abnormalities or infection.
- HIV ELISA negative

ii) **Non-haemodialysing CRF:**

- Creatinine clearance of 5-50 ml/min (approximately plasma creatinine 150-1500 μmol/l).
- Clinically stable patient
- HIV ELISA Negative

iii) ESRD on haemodialysis:

- Creatinine clearance of less than 5ml/min (32) (approximately plasma creatinine > 1500 μmol/l).
- HIV ELISA Negative

i) Inclusion Criteria

- Age 12-60 years; due to a low incidence of CRF below 12 years and a decrease in creatinine clearance above 60 years (33).
- either be a normal person for control purpose, non-haemodialysing CRF or ESRD on haemodialysis.

ii) Exclusion Criteria

- Clinical evidence of infection that would interfere with baseline immunological parameters
- History of ingestion of drugs known to interfere with immune system, eg steroids, cytotoxics, antithyroid drugs, cigarette smoking and alcohol.
- Illnesses known to interfere with immune system like, HIV infection, diabetes mellitus, leukemia, primary immune deficiencies.

Laboratory investigations

These included:

1) Haemogram:

2 ml of blood in a sequestrene bottle was taken for WBC count and differential by a coulter counter model S plus iv and v.

2) Serum creatinine:

3 ml of blood was taken in a plain bottle.

Serum was separated from it by letting it stand for 30 minutes. To determine the amount of creatinine, the serum was reacted with picric acid to give a red coloured solution whose absorbency was read by calorimetric method in an Autoanalyser.

Cockcroft and Gault formula was thereafter applied to calculate the creatinine clearance (34).

$$\text{Creatinine clearance} = \frac{1.2 \times (140 - \text{Age yrs}) \times \text{weight (Kg)}}{\text{plasma creatinine (micromol/litre)}}$$

(Reduced by 15% for women)

3) HIV ELISA:

2ml of blood in a plain bottle was taken for the detection of HIV antibodies by ELISA. This method was by

Enzygnost Anti-HIV-1 / HIV-2 kit by Paul - Ehrlich - Institute (Germany) (35).

Procedure:

Wells on prewashed test plate were filled with 0.3 ml of sample buffer. Test samples were then added into the wells, including both positive and negative controls. The plate was then sealed with adhesive foil and incubated at 37°C for 30 minutes. The foil was then removed and all wells aspirated and washed 4 times with washing solution. The wells were then filled with 100 µl of working conjugate dilution and incubated as before. The foil was then removed and the wells washed 4 times as before. 100µl of washing chromogen solution was then added into each well and incubated at 15 to 18°C for 30 minutes, protected from light. The reaction was then stopped by adding 100 µl of stopping solution POD to each well and left for 30 minutes. The solution was then read at 450 nm. Sensitivity of the test for HIV positive was 100%. Specificity for normal negatives ranged from 95.05 - 100%.

The second aspect of laboratory investigations was carried out to determine the immunological parameters in the cases and controls blood samples were taken for:

1) **CD4+ and CD8+ T-lymphocyte count:**

2ml of blood was taken (before dialysis in ESRD) in a sequestrene bottle for T-cell subset enumeration by flow cytometry using a FAC Scan-BD model.

Procedure:

100 μl of the whole blood was mixed with 20 μl of antibody solution and incubated at $4^{o}C$ for 30 minutes. 2 ml of FACS lysing solution was then added and the mixture incubated in the dark for 15 minutes. The mixture was then centrifuged at 1500 rpm for 5 minutes and thereafter the supernatant poured out. The residue was then washed with 2 ml isoton solution and centrifuged again at 1500rpm for 5 minutes and the supernatant poured out. The residue containing the cells was then fixed with 0.1% paraformaldehyde and analysed. CD4+ and CD8+ T-lymphocytes were stained differently by specific monoclonals and analysed using simulset software.

2) **Immunoglobulin Concentration:**

2 ml of blood was taken in a plain bottle before dialysis and immunoglobulin concentration determined by method developed by Mancini et al (36).

Melted 3% agar gel buffered at pH 8.6 was mixed with an approximate amount of specific antiserum and poured as a 1 mm thick layer. After the agar had set, wells of 1 mm

diameter were punched out. An accurately measured volume of antigen was introduced into each of the wells by means of a micro-pipette. After incubating in a damp atmosphere for 24 hours, the diameter of the precipitate ring was measured after migration had stopped. A series of dilutions of standard antigen was set up as a reference on the test plate. For a given concentration of the antigen (in the well), the diameter of the precipitate ring bears a linear relationship to the initial concentration of the antiserum (in the plate).

This method has been adopted for the quantitative estimation of different classes of immunoglobulin in serum and other body fluids employing antisera specific for the heavy chains of immunoglobulin, in this study IgG,IgA and IgM. It is most accurate for the estimation of low levels of immunoglobulin, as in the case of serum from subjects with hypogammaglobulinaemia.

Data analysis

The data was entered and analyzed using the SPSS/SC+ package. Since the data did not conform to a normal distribution, a non parametric analysis was done.

Descriptive statistics

The mean cell counts of CD4+ and CD8+ of the patients with ESRD on haemodialysis and non-haemodialysing CRF were computed. Similar computations were performed for the control group. Evaluation of mean serum concentration of IgG, IgA and IgM in the different groups were done. Corresponding confidence interval for these groups of renal conditions were constructed to provide an estimate of the range of population values for the variables measured.

Comparison statistics

The mean CD4+, CD8+ T-lymphocyte count and serum immunoglobulin concentration in a given group were compared with the control.

Associations

Association between the variables in the study were investigated using the Chi-square test in the case of the cell counts. The spearmans product moment correlation is applied in the case of serum concentration of the immunoglobulins.

Presentation

The results from the analysis were presented in both tabular and graphical form.

RESULTS

The study was carried out between June and October 1996 at Kenyatta National Hospital renal unit, medical wards and blood donor centre. A total of 90 individuals who fulfilled the inclusion criteria were recruited. This comprised of 30 cases of ESRD on haemodialysis, 30 non-haemodialysing CRF and 30 normal controls.

AGE AND SEX DISTRIBUTION

The study subjects ranged from 13 to 60 years with a mean of 31±(SD) 13 years. The age distribution in the 3 groups were found to be similar with no statistically significant difference ($p>0.05$).

Table 1: Age distribution in the various study groups

	Age (Years)		
	Mean	Standard deviation	Cases
ESRD	23	10	30
NON HAEMODIALYSING CRF	39	16	30
CONTROL	31	8	30

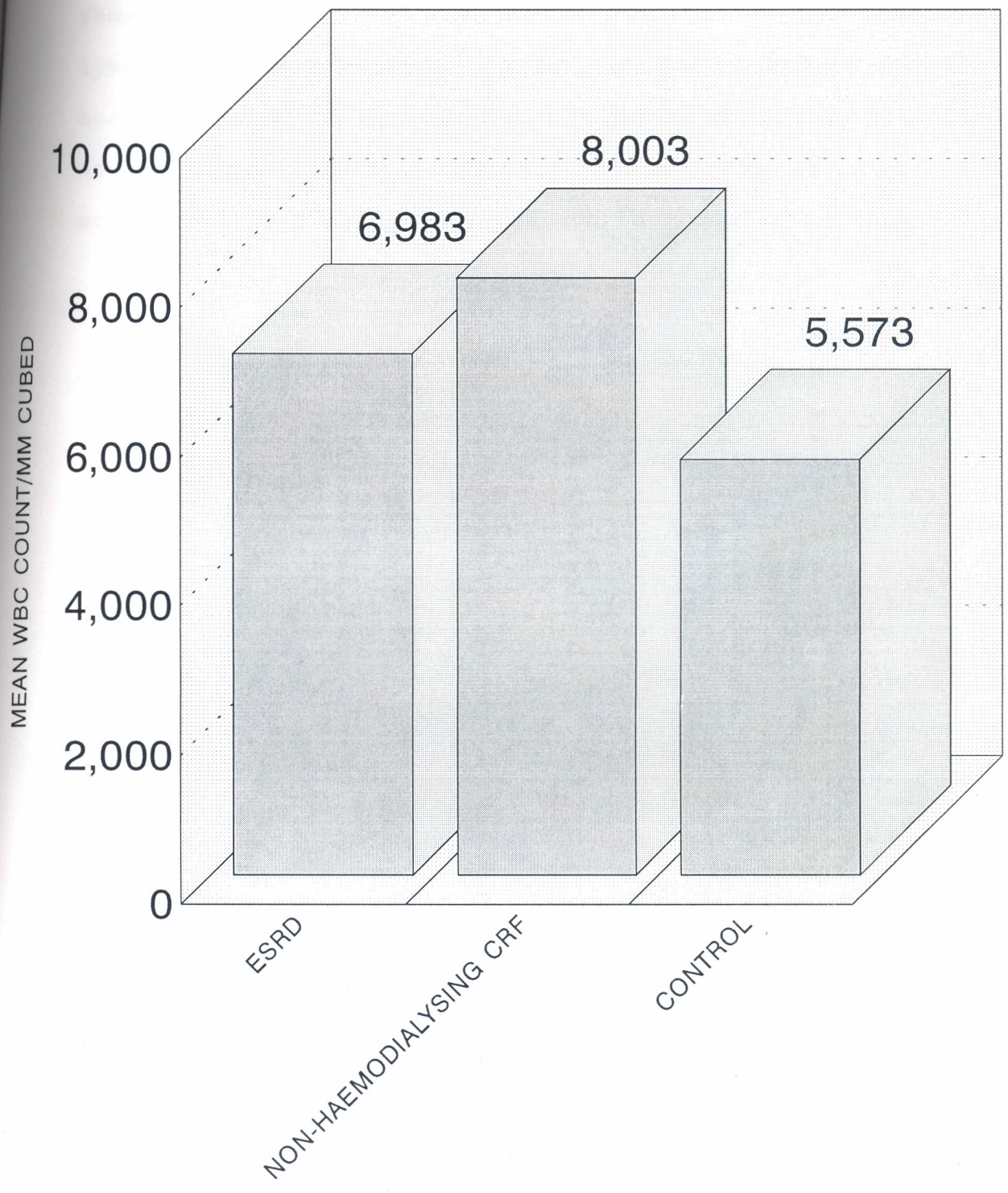
A total of 48 males and 42 females were investigated. The sex distribution in the various study groups was as shown in Table 2.

Table 2: Sex distribution in the various study groups

	SEX		No. of Cases
	Male	Female	
ESRD	18	12	30
NON HAEMODIALYSING CRF	14	16	30
CONTROL	16	14	30
TOTAL	48	42	90

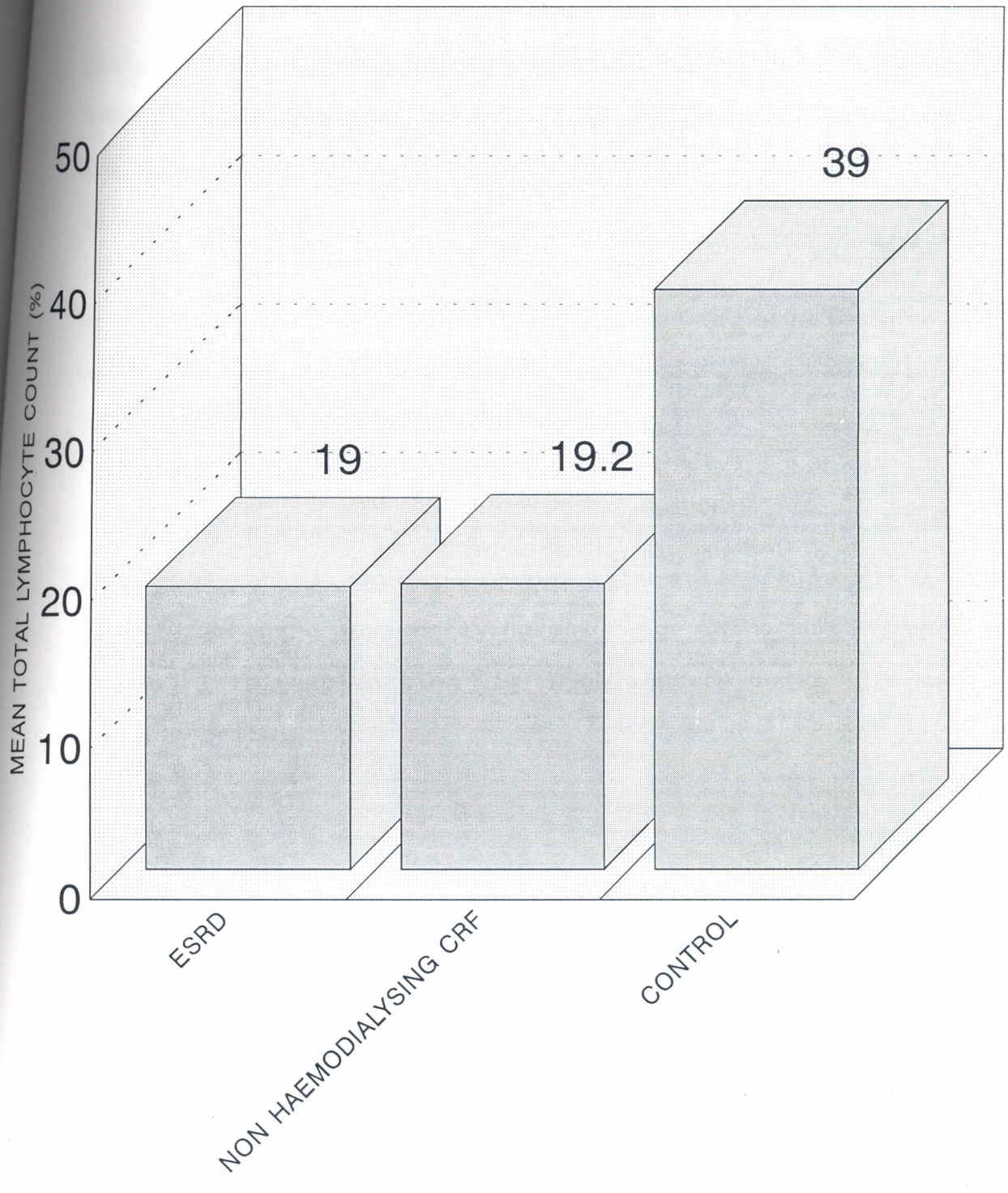
TOTAL WHITE BLOOD CELL COUNT:

Figure 1 shows the mean WBC count in the various study groups. The total WBC count in the 3 groups showed no statistically significant difference in absolute values ($p>0.05$). The counts were within the normal range both in uraemic patients and in the normal controls.



STANDARD DEVIATION
ESRD - 4590, NON-HAEMODIALYSING CRF - 1335, CONTROL - 1376
FIGURE 1 - Mean WBC count in the study groups

The total lymphocyte counts in the various study group are shown in Figure 2. Statistically significant low levels of lymphocytes were observed in ESRD on haemodialysis and non-haemodialysing CRF compared to the normal controls ($p<0.001$). No statistically significant difference was noted between the non-haemodialysing CRF and ESRD on haemodialysis ($p>0.05$).



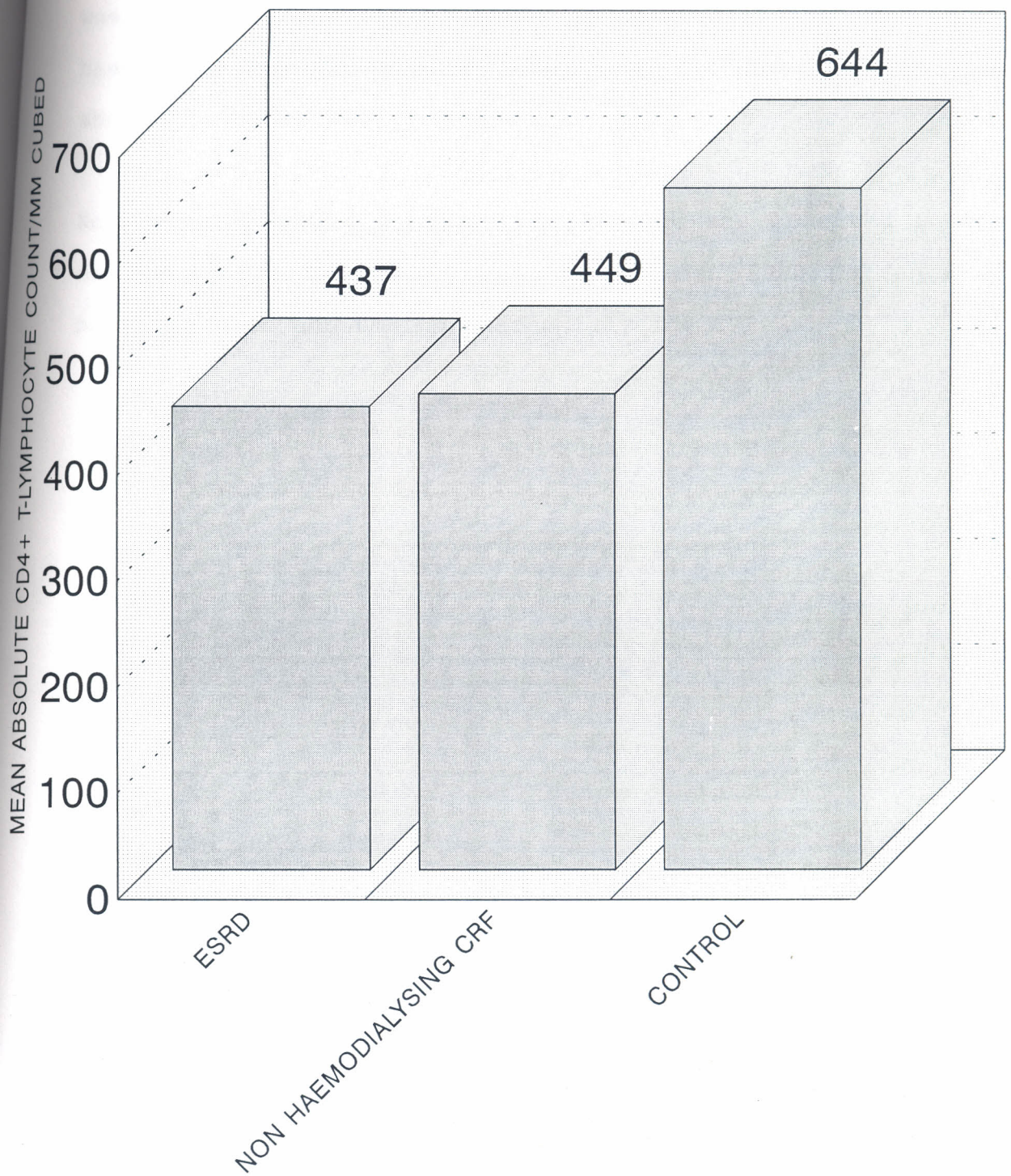
TANDARD DEVIATION
SRD - 14.0, NON-HAEMODIALYSING CRF - 9.0, CONTROL - 11.0
gure 2 - Mean total lymphocyte count in the study groups

T-CELL SUBSET

Statistically significant reduction in absolute CD4+ T-lymphocyte count was found in ESRD patients on haemodiaysis and non-haemodialysing CRF as compared to the normal control as shown in Figure 3 ($p<0.05$).

No statistically significant difference was noted between non-haemodialysing CRF patients and ESRD on haemodialysis ($p>0.05$).

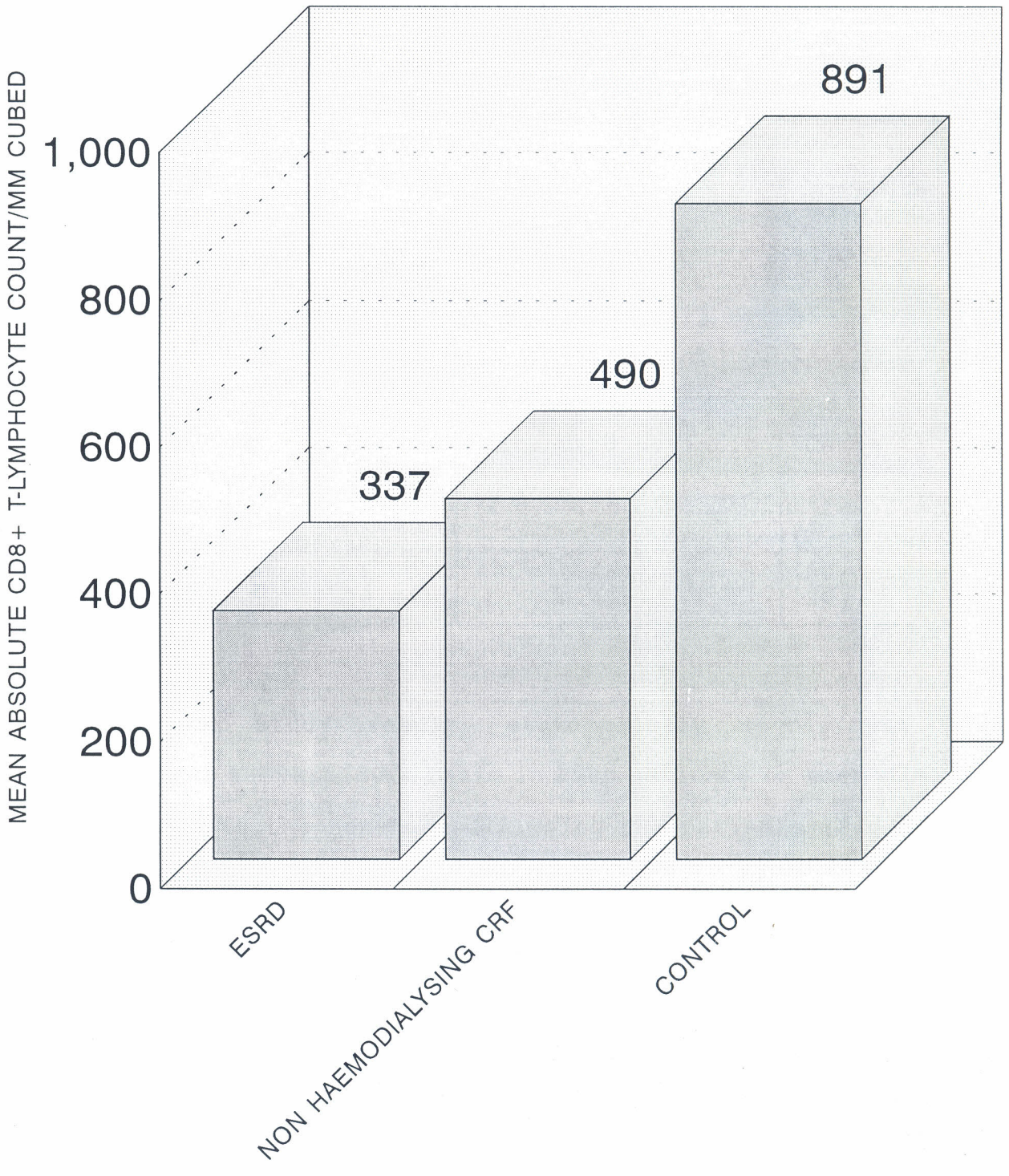
A low mean absolute CD4+ T-lymphocyte count was noted in the apparently normal control group. This still falls within the locally observed apparently normal range using the FAC Scan BD model. This explanation is highlighted under discussion section below.



NDARD DEVIATION
D - 234, NON-HAEMODIALYSING CRF - 315, CONTROL - 228
ıre 3 - Mean CD4+ T-lymphocyte count in the study groups

Statistically significant low absolute CD8+ T-lymphocyte count was observed in both ESRD on haemodialysis and non-haemodialysing CRF patients compared to the normal controls as shown in Figure 4 ($p<0.001$).

No statistically significant difference was observed in the absolute CD8+ T-lymphocyte count between ESRD patients on haemodialysis and the non-haemodialysing CRF.



TANDARD DEVIATION
SRD - 187, NON-HAEMODIALYSING CRF - 336, CONTROL - 360
gure 4 - Mean CD8+ T-lymphocyte count in the study groups

Table 3 shows comparison of the CD4+:CD8+ T-lymphocyte ratio. Statistically significant high CD4+:CD8+ T-lymphocyte ratio was observed in ESRD patients on haemodialysis compared to the normal control, ($p<0.001$). No statistically significant difference was noted between non-haemodialysing CRF and the control group ($p>0.05$).

Table 3: Comparison of the CD4+:CD8+ cell ratio

	CD4+:CD8+ RATIO		
	Mean	Standard deviation	Cases
ESRD	1.426	0.497	30
NON HAEMODIALYSING CRF	1.093	0.484	30
CONTROL	0.843	0.445	30

From our study result, statistically significant high CD4+:CD8+ T-lymphocyte ratio in ESRD patients on haemodialysis compared to the non-haemodialysing CRF patients ($P<0.05$) was observed.

The CD4+:CD8+ T-lymphocyte of the apparently normal control group is lower than the expected ratio of 1. This however still falls within our normal local range of 0.6-1.6 using FAC Scan BD model. Possible explanation of this has been highlighted under dicussion section.

SERUM IMMUNOGLOBULIN ASSAY

Table 4 shows serum concentration of immunoglobulins in ESRD, non-haemodialysing CRF and control group as calculated by comparison statistics against standard normal serum.

Table 4: Serum immunoglobulin concentration in the study groups.

Mean	ESRD	NON HAEMODIA-LYSING CRF	Control	p-value
IgG; Diameter (mm)	10.10	10.07	9.60	0.1197
Concentration(%)	91.97	91.30	87.40	0.1241
IgM; Diameter (mm)	6.03	6.03	6.17	0.8377
Concentration(%)	79.03	75.60	77.30	0.8285
IgA; Diameter (mm)	9.70	8.83	9.13	0.3361
Concentration(%)	88.43	80.47	83.27	0.3357

No statistically significant difference in serum concentration of IgG, IgA and IgM was noted in patients with ESRD on haemodialysis, non-haemodialysing CRF and the control group ($P>0.05$).

DISCUSSION

Introduction

Immunodeficiency is a well established finding in uraemic patients (1,2). This is reflected by the increased susceptibility to infections, increased incidence of cancer, burn-out of immunological disorders and inadequate response to antigenic challenge.

Lymphocyte response:

Statistically significant lymphopenia and low absolute CD4+ and CD8+ T-lymphocyte were observed in ESRD on haemodialysis and non-haemodialysing CRF. Similar observations have been made in previous studies (4,12,21,22,26,28,37,38). Relatively low absolute CD4+ T-lymphocyte count was observed in the normal control group, with a mean of 644±228. This is in keeping with the available local figures for apparently normal individuals using FAC Scan BD model. The range has been 380-1080/mm^3 (see Appendix D). Several confounding factors may be attributed to this, most of which were apparently taken care of in our study. These include; malnutrition, infections, diabetes mellitus, immunosuppressive drugs (e.g steroids, cytototics, antithyroid, cigarette smoking, alcohol etc).

Lymphocytes are either thymus derived (T-lymphocytes) or bone-marrow derived or bursa-equivalent (B-lymphocytes), both of which originate from a common stem cell. Lymphocytes are

amongst the principal effector and regulator cells of the immune system. Profound lymphopenia observed in uraemic patients therefore result in both cellular and humoral immune deficiency.

From the study done by Barsotti et al (19), the inhibitory capacity of uraemic sera was found to have both dialyzable low molecular weight urea and creatinine which happen not to be toxic to lymphocytes, and high molecular weight methyl guanidine which is non dialyzable and toxic to lymphocyte. This observation may partially explain the lymphopenia observed.

Uraemic patients therefore are susceptible to a whole range of infectious agents including organisms not ordinarily considered pathogenic. Multiple infections with viruses, bacteria and fungi occur, often simultaneously. Because donor lymphocytes cannot be rejected by these recipients, blood transfusions can produce fatal graft-versus-host disease. Neoplasms have also been increasingly observed (21).

Our study revealed significantly high CD4+ : CD8+ T-lymphocyte ratio in ESRD patients on hemodialysis compared to the non-haemodialysing CRF patients. Conflicting reports of CD4+ : CD8+ T-lymphocyte ratio have been noted. Reports of increased (28), normal (3,13,26) and reduced (21,24) ratio exist. The

local normal range of CD4+:CD8+ T-lymphocyte ratio is 0.6-1.6 using FAC Scan BD model (see Appendix D). From our study, the normal control individuals had CD4+:CD8+ T-lymphocyte ratio of 0.843. This falls within our normal local range but low compared to the expected ratio of 1.0 in the normal individuals. Local confounding factors causing immunosuppression (already discussed) may be attributed to this. In our study however, most of these factors were taken care of.

The higher CD4+:CD8+ T-lymphocyte ratio observed in ESRD patients on hemodialysis implies that they have better cellular immunity than the non-haemodialysing CRF. Clinically there may not be significant difference. However, data are now emerging rapidly in favour of the hypothesis that uraemia is associated per se with a state of cell activation (13,22). Higher activated T-cell counts (T-cells with DR antigen and Interleukin 2R) have been observed in uraemic patients treated with hemodialysis and continuous ambulatory peritoneal dialysis (28,29). These results provide evidence that ESRD, apart from altered proportional and absolute values of the different immune cell subsets, is characterized by a state of immune activation. The paradoxical association between immunodeficiency and clear-cut signs of cell activation provokes the search of immune activating compounds in uraemia (40).

Humoral response

The serum concentration of Immunoglobulin G, A and M did not show statistically significant difference between ESRD on hemodialysis and non-haemodialysing CRF compared to the normal controls.

Uraemia per se, does not seem to affect the serum concentration of IgG, IgA and IgM since the control group and the non-haemodialysing CRF patients had similar concentrations from our study. This may be explained indirectly by studies on B-cells.

In our study, we did not look at the B-cells, but previous quantitative studies on hemodialysis patients showed significantly low B-cell numbers (11,20,28,31,32,38). Despite the low absolute B-cell count observed however, uraemic B-lymphocyte have been shown to produce abnormally high IgM and IgG titres in absence of stimulation (E.Rothschild and L.Chatenoud, unpublished data). These could explain the normal serum concentration of IgG and M and presumably IgA that is observed in our study.

The normal serum concentration observed of the IgG, A and M however does not reflect the true state of humoral immunity in the body. This is because specific in-vivo and in-vitro

antibody responses are significantly impaired in uraemic patients. It is not clear whether the defect is intrinsic to B-lymphocytes or linked to a defect in T-B cell cooperation, since T-cells produce several lymphokines that are fundamental for B-cell differentiation (29). As a result, uraemic patients respond poorly to antigen challenge e.g. against hepatitis B-vaccine, BCG vaccine etc. The immunologic disorders observed in certain disease conditions have been noted to burn-out when the individual become uraemic. All these support the poor antibody response observed in uraemic despite the apparently normal serum concentration of the immunoglobulins.

CONCLUSION

Non-haemodialysing CRF patients and ESRD patients on hemodialysis have lymphopenia, low absolute CD4+ and CD8+ T-lymphocyte count, and normal serum concentration of IgG, A and M. They show varying CD4+ : CD8+ T-lymphocyte ratio, which is higher in ESRD patients on hemodialysis.

It is therefore concluded that quantitative alteration of the immune cells, especially T-cells exist in non-haemodialysing CRF and ESRD on hemodialysis and account for at least in part, the immune dysregulation associated with chronic renal failure.

STUDY LIMITATION

The study did not take into account all the confounding factors that may affect the CD4+ T-lymphocyte count. These are many and it may not be possible to take into account all these factors. The factors include: smoking, alcohol, infection, connective tissue disorder, malnutrition, primary immune deficiencies, diabetes, malignancies and diurnal rhythm of ACTH. In our study, we did not adequately take care of diabetes in the control individuals since we didn't do blood sugars on them, primary immune deficiencies, connective tissue disorders and malignancies. This was due the short time limit for the study and financial constraint. A multivariant analysis would have been better in our analysis but was not possible for the same reason.

RECOMMENDATION

1) The precise mechanism of immunopathology in uraemic patients need to be fully elucidated so that definite intervention to counter the dearrangement(s) can be worked out. Alot more work is still needed in this area.

2) More research is needed to identify the suppressor substance(s) in uraemic patients and ways of removing them from the body. Currently low molecular weight toxins like urea and creatinine are the only dialysable substances isolated. More toxic high molecular weight substance like Methyl guanidine which severely impair lymphocyte blastogenesis at concentration similar to those found in uraemic serum cannot be dialysed (17).

3) The observed paradoxical association between immune deficiency and clear cut signs of B-cell activation under basal condition (40) provokes a search for the immune activating compounds in uraemia. From our study, the serum concentration of IgG, IgA and IgM did not show statistically significant difference between the uraemic patients and the normal control group despite low number of B-lymphocytes observed in haemodialysis patients with ESRD (20,28). Research is still needed in this area.

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APPENDIX A

Consent Form

You have been invited to participate in this study that analyses some immunological parameters in uraemic patients and kindly requested to participate as you have a renal disease causing uraemia. You'll be asked a few questions and clinically examined. Blood specimen will be taken from you for laboratory analysis and the results discussed with you. All information concerning you and the laboratory results will be kept safely and treated with a high degree of confidentiality by the investigators.

Patient signature .....

Signature of witness .....

APPENDIX B - PROFORMA

Date _____

Number _____

Case particulars

Name _____ IP/OP/No _____

Sex _____ Clinic/Ward /Unit _____

Age (yrs) _____

Weight (kgs) _____

Laboratory Results

1) Haemogram:

WBC Count _____

Differential Count _____

2) Serum Creatinine _____ μmol/l

Creatinine Clearance _____ m1/min.

3) H I V ELISA _____

4) Lymphocyte Differential:

Total count_____

CD_4 Count _____

CD_8 Count _____

5) Serum immunoglobulin Concentration:

Ig A _____

Ig G _____

Ig M _____

Diagnosis of the patient _____

APPENDIX C - CONTROLS PROFORMA

Date _____

Number _____

Case Particulars

Name _____

Sex _____

Age (yrs) _____

Weight (Kgs) _____

1) Haemogram:

WBC Count _____

Differential Count _____

2) Serum Creatinine _____ μmol/l

Creatinine Clearance _____ ml/min.

3) H I V ELISA _____

4) Lymphocyte:

Total count _____

CD_4 Count _____

CD_8 Count _____

5) Serum immunoglobulin Concentration:

Ig A _____

Ig G _____

Ig M _____

APPENDIX D - LYMPHOCYTE DIFFERENTIAL COUNT

LOCAL NORMAL RANGES USING FAC-SCAN BD MODEL

Lymphocyte % 22-45%

CD_4 % 31-44%

CD_8 % 28-48%

CD4 Absoulte 380-1080/cumm

CD8 Absolute 370-1010/cumm

Ratio CD4/CD8 .....0.6-1.6

CHILDREN < 2YRS .....1053-2787/cumm (mean 1920)

CHILDREN > 2YRS .....627-2009/cumm (mean 1318)

PRINTED DATA FROM W.H.O. CENTRE FOR RESEARCH AND TRAINING ON AIDS AND STD; DEPARTMENT OF MEDICAL MICROBIOLOGY, UNIVERSITY OF NAIROBI.