BIOCHEMICAL CHANGES IN URINARY TRACT INFECTIONS

by

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INTRODUCTION

I INTRODUCTION

Urinary tract infection results when the urinary tract of susceptible host is invaded by virulent bacteria usually E.coli (Ronald,1981). Urinary infection is of greatest frequency in the early years of life because it so commonly complicates infection elsewhere particularly in the respiratory and gastrointestinal tracts. Urinary tract infection is second in frequency to upper respiratory infection. According to Chakraborty et al., (1972). The incidence runs as high as 20-25 per cent of the population.

Urinary tract infection is a common urological condition and the diagnosis cannot be made without the bacterialogical examination of urine. Patients with classic symptoms of urinary infection may have a sterile urine and asymptomatic may have a significant bacteriuria (Engel et al., 1980).

Vesico - urethral reflex and attempt of instrumentation in urinary tract may enhance spread of infection. Urinary tract infection is most common disorder at all ages and in both sexes. However urinary tract infection is more common in females because of the anatomy of genito urinary system and in older males because of enlarged prostate (Doifode et al., 1982).

Urinary tract infection is a common cause of morbidity and mortality in our community and is responsible for prolonging the days of hospitalization in surgical patients (Singh et al., 1981). Not only is the condition disabling but recurrence is a common feature and chronic infection can lead to dangerous sequale like hypertension and renal failure. Besides the clinically manifest disease 'silent' infections also occur. Their diagnosis is difficult because the voided urine is not sterile and more presence of micro organisms is no proof of urinary tract infection (Kamet et al., 1980). Acute infections of the urinary tract are relatively common in paediatric practice. Frequently such infections turn into latent pyelonephritis with many occasionally flare - ups. These lead to steadily advancing disease ending in renal failure, uraemia and death (Purwar et al., 1972).

The prevalence rates of bacteriuria during the first trimester pregnancy has been reported to be in the range of 4 - 10 per cent (Kass et al., 1956). However the prevalence of bacteriuria is also as frequent in nonpregnant married women of the same age (Tomlinson et al., 1969). In children the prevalence has been

reported to be in the range of 1.5 to 2.4 per cent (Doherty et al., 1968). Urinary tract infection occurs more than twice as often during the first twenty four months of life than in any similar period in the succeeding years of childhood (Campbell, 1970). Turner (1961) belive that infection rate depends on the sexual activity of female subjects. At the other end of life urinary tract infections are exceedingly common reaching about 30 per cent in admissions to geriatric hospitals (Mukerjee, 1976).

Urinary tract infection was defined as greater than 100,000 organisms per CC in a clean catch mid stream or catheterized urine specimen or greater than 10,000 organisms in atleast three consecutive specimens (Krieger et al., 1980). However under certain conditions the bacterial count may be low even in significant bacteriuria, specially in patients receiving antibiotic therapy and in some coccal infections (Chakraborty et al., 1972).

E. Coli is the commonest organism isolated in almost all studies. The other bacteria such as Streptococcus faecalis, Streptococci, Staphylococci,

Klebsiela, Proteus species, Achromobacter and Pseudomonas are less common infections contracted outside the hospital (Gulati et al., 1981). In some of the cases of recurrent infection, a different organism or a different serotype of the same organism is found. The end stage of the bacterial infection of the urinary tract results in chronic pyelonephritis. It may be difficult to diagnose this condition as many patients may have a sterile protein free urine with abnormal white cell excretion (Mukerjee,1976). Staphylococcus saprophyticus urinary infection is more common in young females in the age group of 16-25 years but rare among men and among hospitalized patients of both sexes (Vinod Kumar et al., 1982).

The patients with urinary tract infections are symptomatic because of renal parenchymal involvement. It is important to distinguish between kidney and bladder infection. Patients with renal infection have decreased renal function, increased antibody and antibody coated bacteria in urine. Recurrence with the same organism are common and short term antibiotic therapy is not effective. On the otherhand patients with bladder infection have a normal concentrating ability. The antibody titer is negative and antibody coated bacteria are not

recovered from the urine.. Recurrent infection is unusual but reinfection may occur with a new organism. The pregnant women with renal infection may have premature deliveries but there is also evidence that appropriate treatment doesnot decrease the incidence of prematurity(Osofsky et al., 1978).

Diagnosis of urinary tract infectionis correctly done at the earliest possible time not only saves the expansive modern therapy but also prevents further damage of the urinary system. A wrong therapy may also lead sensitive organisms to become resistant thus endangering life with serious consequences (Patnaik et al., 1983). The problem of urinary tract infection in the young merits special consideration notably when the disease neither disappears spontaneously nor readily responds to treatment (Campbell, 1970).

In normal individuals the pH values of urine is between 5.5 to 6.5 and is acidic. The quantity of phosphorus excreted varies with dietery intake. Since sodium and potassium are major cations of the diet they are also the major cations of normal urine. Chloride is the chief anion of urine and the amount excreted is approximately equal to the amount that has been ingested. The

daily urinary excretion of calcium varies between 0.1 and 0.3 g and it varies with intake of diet. Excretion of urea is a direct function of total nitrogen intake and urinary creatinine bears a direct relation to the muscle mass of individual. Creatinine excretion occurs more regularly in young children than in adults. Normal urine contains traces of protein (including serum albumin and globulins) glycoprotein from the lining of the genitourinary tract and mucoproteins of other origin White et al., 1978)

In urinary tract infection the pH of the urine changes depending upon the organisms. In more acute disturbances of renal function urinary excretion of urea may be diminished leading to more rapid increase in serum urea. Plasma creatinine increases in renal disease. Urinary tract infection associated with stone has increased phosphorus and calcium. In renal disease or other diseases affecting renal function excretion of protein is increased as a result of changes in glomeruli allowing increased passage of proteins. Haemoglobin is not present in normal urine. One ofthe most important causes for the heamaturia is the acute and chronic urinary tract infection. The present investigation was undertaken to study all these parameters in urine and serum of patients

of urinary tract infection. It is hoped that the findings would be of diagnostic value and help in the proper treatment of urinary tract infection.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Review of literature pertaining to the study on "Biochemical changes in urinary tract infections" is discussed under the followig headings:

A. Prevalence of urinary tract infection.
B. Types of urinary tract infection.
C. Symptoms of urinary tract infection
D. Source of urinary tract infection.
E. Laboratory diagnosis of urinary tract infection.
F. Biochemical analysis of urinary tract infection.
G. Urinary tract infection in pregnancy.
H. Urinary tract stones following infection.

A. Prevalence of Urinary tract infection:

Urinary tract infection occurs in both males and females. Reported incidence of urinary tract infection has varied from 0.99 per cent to 2.8 per cent in males and from 1.2 to 4.6 per cent in females. Males do not constitute a major proportion of bacteriuria. A 0.03 per cent to 1.15 per cent involvement of males was seen in different series while a prevalence of 3.5 to 4.6 per cent of bacteriuria was reported of females. (Ghalaut et al., 1982)

Urinary infection occurs more than twice as often during the first twenty four months of life than in any similar period in the succeding years of childhood and

comprises 1 to 3 per cent of the problems of pediatric practice. Urinary tract infection outranks other bacterial disease in children under two years of age (Campbell, 1970).

The common age incidence of urinary tract infection in adult is 21 to 40 years. Female sex predominates over male sex up to the age of 40 years after which male sex predominates over female. The increased incidence of urinary tract infection among females below 40 years of age has been attributed to factors like sexual intercourse and childbirth. Similarly in males above the age of 40 prostatic enlargements, stricture urethra and malignancy have been reported as common factors (Patnaik et al., 1983). In older males senile changes such as prostatic enlargement causing chronic obstruction and stasis of urine play a part and in females post monopausal age runs a special risk of urinary tract infection (Ghalaut et al., 1982). Females in reporoductive age (20 - 40 years) and males, children below ten years and older persons above fifty years were most frequently involved (Singh et al., 1981).

B. Types of Urinary tract infection:

Urinary tract infections are classified into three types: 1) Bacterial nonspecific infections of the urinary tract.

2) Bacterial specific infections of the urinary tract.

3) Other parasitic infections of the urinary tract.

1) Nonspecific infections of the urinary tract:

The nonspecific infections of the urinary tract are a group of diseases having similar manifestations and caused by the gram negative rods (eg.E.coli, proteus vulgaris) and gram positive cocci (Staphylococci and streptoccocci) A pure coccal infection may suggest renal stone.

a. Acute pyelonephritis:

In this bacteria can reach the kidney through the blood stream or they may travel up a ureter that has incompetent ureterovesical value. Severe ache over one or both kidneys occur (Svanborg et al., 1979)

b. Chronic Pyelonephritis:

This implies the persistent presence of bacteria in the kidney.Chronic pyelonephitis is usually associated with stones or obstructive lesions of the urinary tract Dodson, 1970).

c. Bacteremic shock:

This is caused by cardiac decompensation inadequate blood volume and enlargement of vascular space.

d. Necrotizing papillitis:

This is an uncommon type of renal inflammation.

e. Renal carbuncle:

It is due to unilateral hematogenous infection complicating a pyogenic skin lesion.

f. Perinephric abscess:

This lies between renal capsule and renal fasica.

g. Non specific infection of the ureter:

Infection may reach the ureter by the descending route from hematogenous renal infection from the baldder or by the ureteral and periureteral lymphatics.

h. Non specific infections of the bladder:

Acute cystitis:

Acute inflammation of the baldder is a quite common complaint particularly with female patients. Symptoms develop 36 - 48 hours after sexual intercourse are burning on urination, nocturia, heamaturia (Roderick et al., 1963)

ii) Chronic cystitis:

It is chronic infection of the bladder often secondary to chronic infection of the upper tract.

i. Nonspecific infections of the prostate gland:

These are the acute prostatitis and prostatic abscess and chronic prostaties.

j) Nonspecific infections of the urethra

k) Nonspecific infections of the epididymis

l. Nonspecific infections of the testis.

2. Specific infections of the urinary tract:

a. Tuberculosis:

It is more common in males than females. The infecting organism is Mycobacterium tuberculosis. It may take 15 - 20 years to destroy kidney in a pattent with good resistance to infection (Harrison et al., 1979)

b. Amicrobic cystitis:

It is a rare disease affects adult men occasionally children usually boys manifested as acute inflamation of the bladder.

c. Actionomycosis:

Actionomycosis is a chronic granulomatous disease involves kidney, bladder or testis by hematogenous invasion from a primary site of infection.

3. Other parasitic infections;

Other parasitic infections of the urinary tract are Trichomoniliasis in male, Schistosomiasis, Filariasis, Echinococcosis (Hydatid disease), Reiter's disease (Smith, 1978).

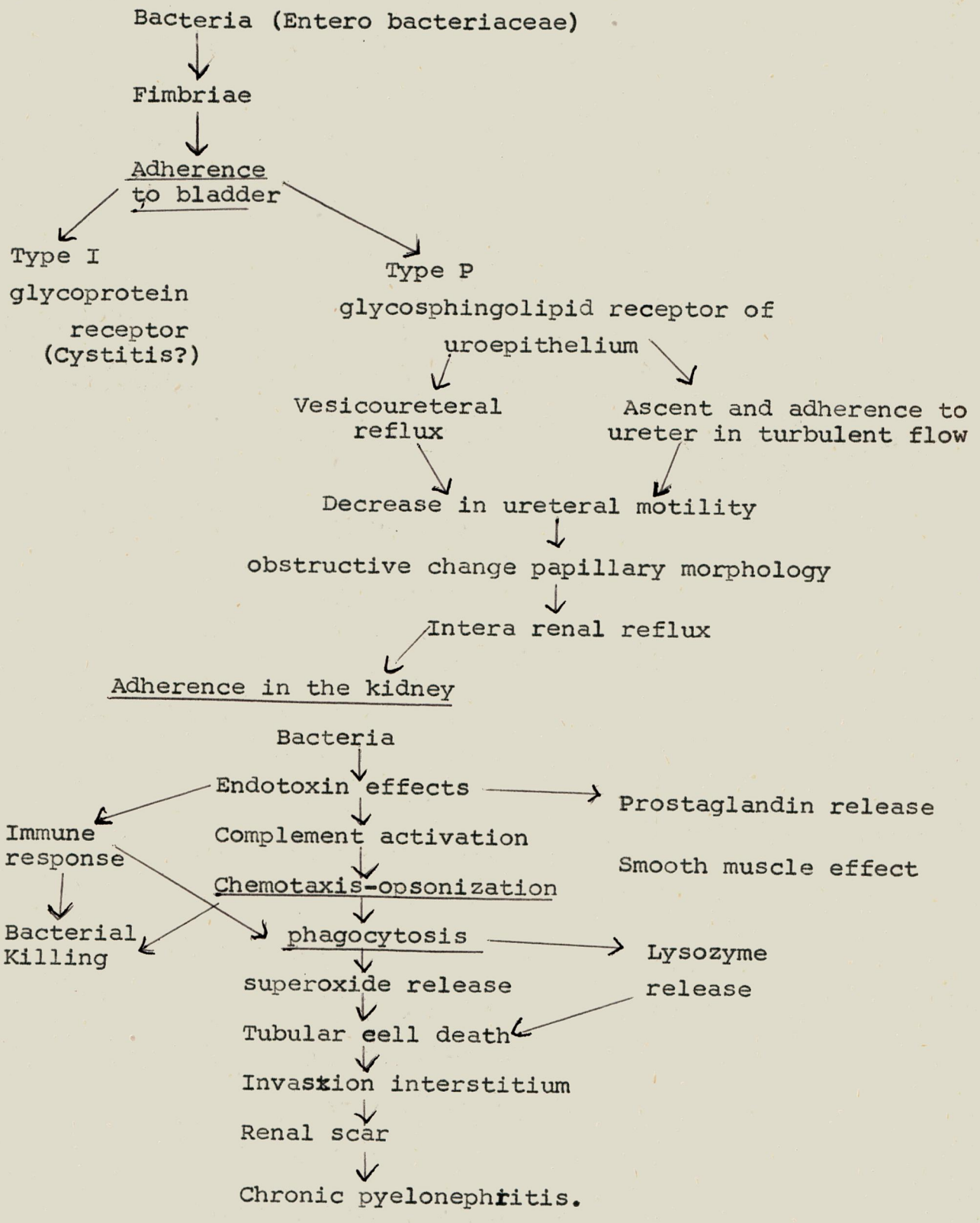
c. Symptoms of urinary tract infection:

Symptoms in adults are pyuria, urinary discomfort, fever, renal type of pain, burning on urination, hematuria, nocturia, neurogenic bladder dysfunction, symptoms in children are abdominal pain, flank pain as tummy ache and high fever. (Bhupendera et al., 1981)

d. Source of urinary tract infection:

An upper respiratory infection may be the initiating source of urinary infection. In the young adult female the infection may be due to improper hygiene, coital trauma, vaginal infections etc. (Smith, 1970) Infections in the female are often initiated by gynecologic disolders, hormone changes and the short urethra. Obstetric trauma gives rise to scarring, cystocele, urothrocele and urethral stenosis which results in urinary obstruction and infection (Roderick et al., 1963)

Pathogenesis of pyelonephritis (Roberts, 1983)



Pyelonephritis has been defined as an infectious disease of the kidney characterised by a primary inflammatory reaction of the pelvis and parenchymal interestices to invading organisms with secondary effects on the tubular, glomerular and Wascular apparatus (Sussman et al., 1967) Chronic pyelonephritis was the commonest disease leading to chronic renal failure followed by chronic glomerulone - phritis (Upadhyay et al., 1980)

e. Laboratory diagnosis of Urinary tract infection:

Precipitation of solutes occurs as a result of bacterial action. Cloudiness in a feshly voided urine may be due to the presence of bacteria or pus in urine. Proteus infections produce an ammoniacal urine and less characteristics odours are produced by infections due to other bacteria (Tietz, 1976).

a. Culture:

Clean voided midstream samples of urine are employed for culture. The sample should be fresh. For quantitative culture serial ten fold dilutions of urine are tested by the pour plate or surface culture methods.

The most widely used method employs a standard loop. One loopful of urine is placed on a non inhibitory medium (blood agar) and another loopful of an indicator medium (Mac.Conkey 1963). The first medium gives a quantitative measurement of bacteria while the latter presumptive diagnosis of the bacterium isolates are identified by their properties.

b. Antibiotic sensitivity tests:

Antibiotic sensitivity tests may be done directly using the urine samples as inocula and the results confirmed by repeating the test with individual isolates.

c. Screening tests:

a. Griless nitrite test:

This is based on the absence of nitrite in normal urine.

b. Catalase test:

The presence of catalase as evidenced by frothing on addition of hydrogen peroxide indicates bacteriuria, tough a positive result is obtained also in hematuria.

c. Triphenyltetrazolium chloride (TTC) test:

Based on the production of a pink - red precipitate in the reagent caused by the respiratory activity of growing bacterial.

d. Microscopic demonstration of bacteria in Gram stained films of urine.

e. Glucose test paper:

Based on the utilisation of the minute amountsof glucose present in normal urine by bacteria causing the infection. (Anantha Narayanan et al., 1982)

Patnaik et al., (1983) have found the following results in patients of urinary tract infection.

	Number of cases	%
1. Pyuria	220	100
2. Haematuria	55	25
3. Casts of varius types	40	18
4. Crystals	23	15
5. Proteinuria	44	20
6. Phosphaturia	12	5.4
7. Bacteriuria	132	60
8. Glycosuria	9	4

F. Biochemical analysis:

i) Determination of urinary excretion of sodium, potassium and chloride:

If kidney function in the body is damaged the ability to conserve sodium, potassium, chloride is frequently lost, kidneys may retain them rather than eliminate. The excess of these causing fluid retention resulting in oedema, severe disordersof sodium, potassium, chloride and water balance can result. It is necessary that high intake of sodium, potassium and chloride in kidney diseases need to be restricted (Antia, 1975) Toxicity of potassium occurs frequently in renal failure when the kidney is not capable of excreting excessive potassium (Harper, 1981). Urinary losses of sodium, potassium arise during recovery from acute obstruction, ~~potassium~~. Renal tubular disease affects the excretion of potassium. This occurs in such cases of chronic pyelonephritis and during recovery from acute renal failure (Varley et al.,1980).

ii. pH:

In the treatment ofurinary infection acid producing drugs are sometimes used to reduce the pH of the urine. Bacterial infections can alter the pH in either direction depending upon and product of bacterial metabolism.

Ammonia producing organisma produce alkaline urine but certain other bacteria will cause the urine to become acid (Tietz, 1970). The three organisms B.Coli the tubercle bacillus and Gonococcus infect the urinary tract commonly and do not split urea. Therefore in these infection the urine is acid. Other organisms however able to split urea. The action of bacterial decompositian is to add water to form Ammonium hydroxide and carbonic acid. Therefore as a result of infection by a powerful urea splitter such as B.Proteus the urine becomes strongly alkaline in reaction and its odour strongly ammoniacal (Parton, 1960)

iii) Urea:

Serum urea naturally can be increased in all forms of kidney disease. In chronic pyelonephritis it may often reach 420 - 720 mg/100 ml. In diseases such as congenital cystic kidneys, renal tuberculosis increases are seen the extent of which depends on the amount of kidney tissue destroyed. In more acute distrurbances of renal function the urinary excretion of urea may be diminished leading to more rapid increase in serum urea (Varley et al., 1980)

Agarwal (1982) in his study of patients with urinary tract infection found the increase of blood urea levels ranging from 41 - 160 mg/100 ml. Studies done by kinacid Smith et al., (1965). found that blood urea was significantly elevated in bacteriuric women.

iv. Creatinine:

Plasma creatinine increases in renal disease. Several workers have reported that the prerenal factors which increase blood urea have little influence on the blood creatinine. Retention of nonprotein nitrogen compounds due to obstruction of urinary tract willcause almost simultaneous and proportional increases inboth urea nitrogen and creatinine levels (Tietz, 1976). Agawwal(1982) found that the serum creatinine values ranging from 1.6 - 5.5 mg/100 ml in patients of urinary tract infection.

v) Phosphorus and calcium:

Phosphate toxicity is rare except when acute or chronic kidney failure prevents normal phosphate excretion (Martin et al.,1981). Urinary tract infection associated with stone has increased phosphorus and calcium. Patnaik et al.,(1983) in his study of urinary tract infection found the increased excretion of phosphorus in urine.

vi. Protein:

The proteins present in normal urine in part are derived from the plasma proteins and in part from the urinary tract. IgA and Tamm Horsfall protein are the urinary tract proteins of special interest. IgA in normal

urine is secretory IgA with antibody activity against E.Coli. so that it may protect the urinary tract against infection. In renal disease or other disease affecting renal function excretion of protein is increased as a result of changes in glomeruli allowing increased passsage of proteins. Proteinuria associated with infection is post renal and is accompanied by pyuria. Proteinuria usually exceeds 5g/day and may even exceed 50g/(Varley et al., 1980).

vii. Haemogolobin:

One of the most important cause for the haematnuria are the acute and chronic urinary tract infection. Most common contributing causes of infection with haematuria is dehydration. Persistent haematuria and pyuria without bacteriuria are the usual initial findings in a patient with urinary tract tuberculosis (Roderick et al., 1970)

H. Urinary tract infection in pregnancy:

Most infections begin with urethral contamination by perineal bacteria which occasionally migrate into the bladder. Factors such as dilation of the ureter, dehydration secondary to vomiting during the first trimester

Gestational glycosuria, aminoaciduria and possibly decreased potassium may predispose the pregnant women to bacteria (Parsons et al ., 1981). The question of when bacteriuria develops or indeed if it develops during pregnancy in women who have no bacteriuria prior to pregnancy has not been answered. It seems likely that since bacteriuria disappears spontaneously within six weeks of delivery in two thirds of women and within six months in more than 80 per cent of patients, most patients are not normally bacteriuric but acquire bacteriuria in early pregnancy and lose it again after pregnancy (Varma et al., 1972)

If bacteriuria present in one pregnancy it is likely to occur in the next in about 50 per cent of patients, where as bacteriuria is very unlikely to develop in a subsequent pregnancy in women who had no bacteriuria the first pregnancy (Mc Fayden et al., 1973)

I. Urinary tract stones following infection:

Role of renal stones in the development of renal failure also in intimately linked to infection. Stones are

composed of calcium phosphate magnesium ammonium sulphate so called triple phsphate stones. Urease produced by Proteus mirabilis and a few species - brakes down urea with the liberation of Ammonia which causes urine pH to increase to struvite and apatite crystals precipitate and stone formation follows (Warren et al., 1981). Stone inurinary tract is cause of wide range of symptoms ranging from general ill health to urinary infection and haematuria (Mathur et al., 1981).

EXPERIMENTAL PROCEDURE

III EXPERIMENTAL PROCEDURE

The experimental procedure adapted for the study on "Biochemical Changes in urinary tract infections" is as follows:

A. Selection of patients

B. Collection of blood

C. Separation of Serum

D. Collectionof Urine

E. Estimation of Biochemical parameters:

i. Creatinine

ii. Urea.

iii. Total protein and albumin-globulin ratio

iv. Phosphorus

v. Calcium

vi. Chloride

vii. Urinary protein

viii. Haemoglonin estimation

ix. Sodium and potassium

A.Selection of patients:

36 persons suffering from urinary tract infection of any type were selected for this study at random from those who attended the outpatient ward of Coimbatore Medical College hospital.

Persons matching in age, sex and who were not suffering from urinary tract infection or any other diseases were chosen as controls.

B. Collection of blood:(Varley et al., 1980)

The blood was collected as follows:

A tourniquet was applied a few cm above the elbow without obliterating the arterial pulse at the wrist. The skin was sterilised over the vein and a disposable sterile needle to a disposable syringe of appropriate capacity usually 10 ml was insterted into the vein. When the needle entered the vein the plunger was withdrawn slightly. The tourniquet was released when the blood appeared. When the desired amount of blood has been drawn ihto the syringe tourniquet was released. The needle was withdrawn. With the needle still in position slowly the blood was transferred to an appropriate container, using the minimum amount of pressure.

C. Separation of Serum:

Blood was transferred to a clean emptytube and allowed to clot for three hours at rpom temperature. The clot was allowed to retract. Then it was centrifuged and the serum was separated using a rubber-bulb pipette. It was collected in a clean dry labelled test tube and stored in the freezer until used.

D. Collection of urine:

Randam samples of urine from patients and control subjects were collected in bottles containing 2.0 ml of Xylene.

E. Estimation of biochemical parameters:

i) Estimation of creatinine:

2.0 ml of serum and 1.0 ml of urine were taken for the estimation of creatinine in serum and in urine colorimetrically by the principle of Jaffe's reaction by Alkaline picrate method (Varley et al., 1980) vide Appendix I

ii) Urea:

0.2 ml of serum and 1.0 ml of urine were taken for the experiment. Urea in serum and urine was estimated colorimetrically by diacetyl monoxime thiosemicarbozide method (Varley et al., 1980) vide Appendix II.

III. Serum protein:

0.4 ml of serum for the determination of total protein and 0.4 ml of serum for the precipitation of globulin were taken and the protein concentration was determined colorimetrically by the method of Biuret (Varley et al., 1980). Vide Appendix III.

iv. Phosphorus:

1.0 ml of urine was taken for the study. The colorimetric estimation of phosphorus in urine was done by the method of Fiske and Subbarow, 1925 (oser, 1976) Vide Appendix IV.

V. Calcium:

2.0 ml of urine was taken for the study. Urine calcium levels were estimated titrimetrically by the method of Clark and Collip, 1925 (Oser, 1976) vide Appendix V.

vi. Chloride:

1.0 ml of urine was taken for the x experiment. Urine chloride level was estimated titrimetrically by the method of Van Slyke. Vide Appendix VI (Varley et al., 1980)

vii. Determination of urinary protein:

2.5 ml ofurine was taken for the experiment. Urinary total protein was determined by the method of Richterich (Varley et al., 1980). Vide Appendix VII.

viii. Haemoglobin:

1.0 ml of urine was taken for the experiment. Haemoglobin present in urine was estimated by Cyanmethaemoglobin method (NIN 1971) vide Appendix VIII.

ix. Sodium and potassium:

Sodium and potassium were determined in Flame photometer (Varley, 1976) vide Appendix IX.

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSIONS

This study was aimed at assessing "Biochemical changes in urinary tract infections". Among the 150 patients who attended the outpatient ward of the Urology section of Coimbatore Medical College Hospital 36 were found to suffer from urinary tract infection and they were selected for this study. The age of the patients ranged between 15 and 85 years. Of the thirty six patients eleven were females and the rest males.

1. Serum and urinary urea, creatinine and protein
2. Urinary calcium, phosphorus and Haemoglobin
3. Urinary sodium potassium and chloride
4. pH of the urine.

The results obtained in this study are discussed under the following headings:

1. Classification of the patients according to the types of organisms that caused infection.
2. Serum levels of urea, creatinine and proteinof patients with urinary tract infection and controls
3. Urinary levels of urea, creatinine and protein of patients with urinary tract infection and controls.
4. Urinary calcium and phosphorus levels in patients with urinary tract infection and controls.

5. Urinary haemoglobin levels in patients with Urinary tract infection.
6. Urinary sodium, potassium and chloride levels in patients with urinary tract infection and controls.
7. Mean pH values of urine in patients with urinary tract infection and controls.
8. Serum albumin - globulin ratio of patients with urinary tract infection and controls.
9. Serum urea, creatinine and protein levels in patients infected with E.coli and in patients infected with other microbes.
10. Urinary urea, creatinine and protein levels in patients infected with E.coli and in patients infected with other microbes.
11. Urinary sodium, potassium and chloride levels in patients infected with E.coli and in patients infected with other microbes.
12. Mean pH values of urine in patients infected with E.coli and in patients infected with other microbes

The patients were classified according to the type of organism that caused infection which is presented in Table - 1.

TABLE 1

CLASSIFICATION OF THE PATIENTS ACCORDING TO THE TYPE OF ORGANISMS THAT CAUSED INFECTION:

Organisms causing infection	Number of patients affected
E. Coli	22
Klebsiela	7
Psedomonas aeruginosa	4
Proteus vulgaris	3
Total	36

Out of the thirty six patients suffering from urinary tract infection twenty two were infected with E.Coli; seven patients had Klebsiela infection; four patients had Pseudomonas aeruginosa infection, and three patients had Proteus vulgaris infection. It was found that infection by E.Coli was more prevalent. Sixty one per cent of the patients had E.Coli infection

Serum levels of urea, creatinine and protein of patients with urinary tract infection and controls are given in Table II and the individual values are given in Appendices IX and X respectively. The serum and urinary protein levels in patients and controls are given in figure I and II respectively.

Figure - 1

MEAN VALUES OF SERUM PROTEIN OF PATIENTS AND CONTROLS

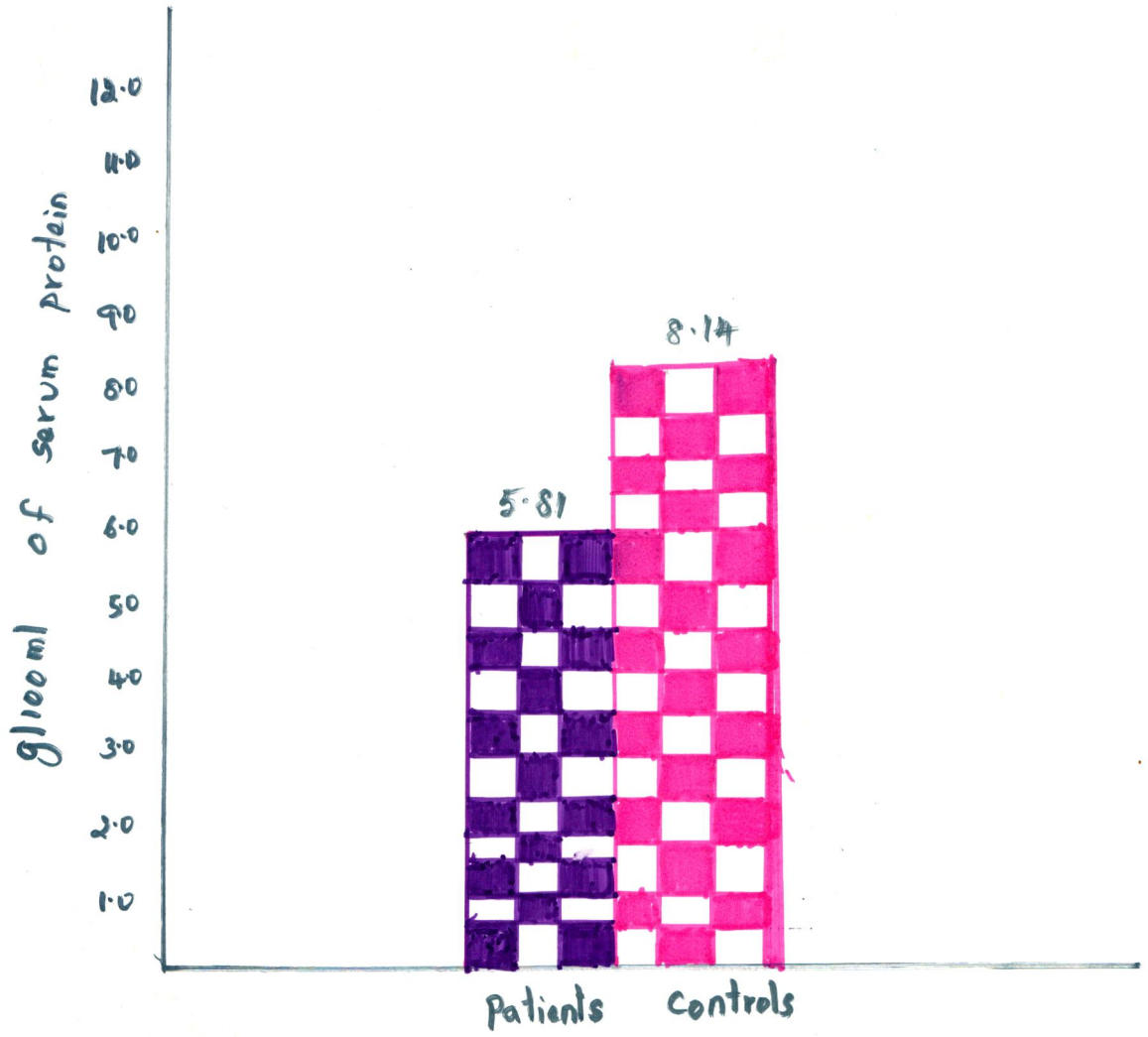


TABLE II

SERUM LEVELS OF UREA, CREATININE AND PROTEIN IN PATIENTS WITH URINARY TRACT INFECTION AND CONTROLS:

Sample	Number	Serum urea mg/100ml Mean±SD	Serum urea mg/100ml Range	Serum creatinine mg/100ml Mean±SD	Serum creatinine mg/100ml Range	Serum protein g/100ml Mean±SD	Serum protein g/100ml Range
Patients	36	72.81± 48.38	24-209	2.93± 1.32	1.9 - 6.60	5.81± 1.51	4.60-6.40
Control	31	33.16± 4.72	24.42	1.85± 0.14	1.60-2.10	8.14± 0.51	7.40-9.20
t value		4.889**		4.873**		8.730**	

** ($P < 0.01$)

As shown in Table II the serum urea levels of the patients ranged from 24 to 209 mg per 100ml, with a mean value of 72.81± 48.38 mg per 100 ml, whereas the mean serum urea level in the controls was found to be 33.16± 4.72 mg per 100 ml with a range of 24 to 42 mg per 100ml. The mean serum urea levels in normal controls were within the range proposed by varley et al., (1980) which is 15-50 mg per 100 ml. The mean serum urea levels ofpatients of urinary tract infection were found to be increased over the control values and this increment was statistically significant ($P < 0.01$).

The mean serum creatinine level in the patients was 2.93 ± 1.32 mg per 100 ml with a range of 1.9 to 6.6 mg per 100 ml whereas the mean serum creatinine level in controls

was 1.85± 0.14 mg per 100 ml with a range 1.6 to 2.1 mg per 100 ml. The serum creatinine level in the controls were found to be within the normal range which is 1.0 to 2.0 mg per 100 ml (Varley et al., 1980). Mean serum creatinine levels of the patients were found to be increased over the control values and this increment was found to be statistically significant ($P < 0.01$).

The mean serum protein value in patients was 5.81± 1.51 g per 100 ml with a range of 4.6 to 6.4 g per 100ml and the mean serum protein value in controls was found to be 8.14 ± 0.51 g per 100 ml with a range of 7.4 to 9.2g per 100 ml. The serum protein values were found to be within the normal range as proposed by Tietz (1976), which is 6.5 to 8.5 g per 100 ml. The serum protein values of the patients were less than the control values and this difference in the serum protein was statistically significant ($P < 0.01$).

All these suggested that in urinary tract infection there is increased serum urea and creatinine which might have been due to thekidney failure. The decreased serum protein may be due to the increased excretion of protein as a result of urinary tract infection.

Figure - 2

MEAN VALUES OF URINARY PROTIEIN IN PATIENTS AND CONTROLS

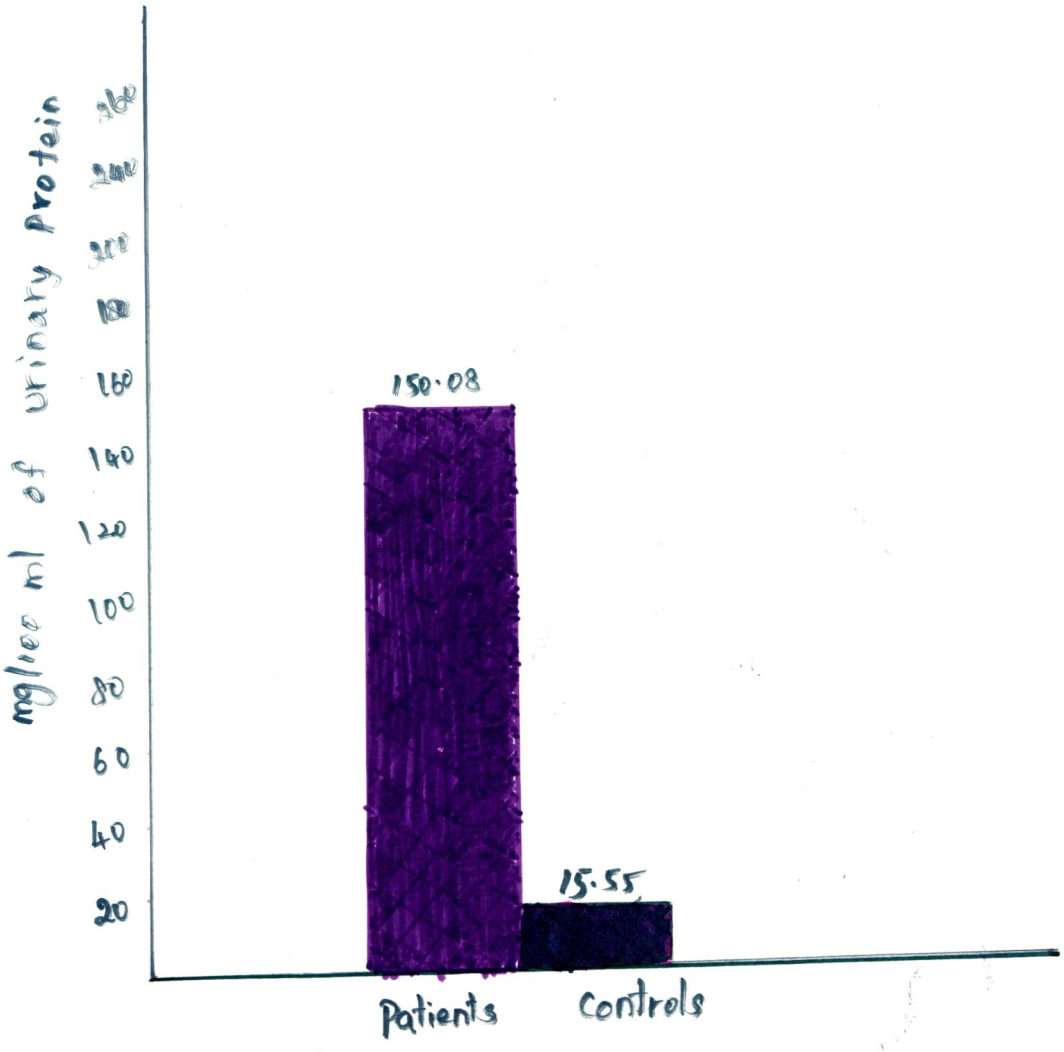


Table III presents urinary levels of urea, creatinine and protein of patients with urinary tract infection and controls. The individual values of urinary urea, creatinine and protein of patients and control are given in the Appendices X and XI respectively.

TABLE III

URINARY LEVELS OF UREA, CREATININE AND PROTEIN OF PATIENTS WITH URINARY TRACT INFECTION AND CONTROLS.

Sample	Number	Urine urea		Urine creatinine		Urine protein	
		g/100ml Mean±SD	g/100ml Range	mg/100ml Mean±SD	mg/100ml Range	mg/100ml Mean±SD	mg/100ml Range
Patients	36	0.93± 0.30	0.59- 1.39	50.28± 17.01	30-92	150.08± 162.26	15-600
Control	31	1.30± 0.11	1.08- 1.46	71.06± 10.52	58-89	15.55± 4.14	8-20
t value		6.882**		6.099**		4.973**	

** ($P < 0.01$).

The mean value of urea excreted in patients was found to be 0.93± 0.30 g per 100 ml with a range of 0.59 to 1.39 g per 100 ml urine whereas the controls the urinary urea ranged from 1.08 to 1.46 g per 100 ml urine with a mean value of 1.30± 0.11 g per 100 ml. Harper (1977) has resported that the normal value of urea excreted is 1.0 - 1.7 g per 100 ml urine Based on this the urea excretion of the control was found to be normal whereas the same was found to be

significantly ($P < 0.01$) decreased in patients with urinary tract infection.

The mean value of creatinine excreted in controls was found to be 71.06± 10.52 mg per 100 ml urine. This is in agreement with the normal range of urinary creatinine exretion which is 55 to 84 mg per 100 ml urine (Harper,1977) the Creatinine excreted by the patients of urinary tract infection ranged from 30 to 90 mg per 100 ml urine with a mean value of 50.28± 17.01 mg per 100ml. The mean value of creatinine excretion in patients was found to be decreased and this decrease was statistically significant ($P < 0.01$)

The mean value of urinary protein was found to be in the range of 8 to 20 mg per 100 ml with a mean 15.55± 4.14 mg per 100 ml urine in controls. In controls the protein excreted in urine is in agreement with the normal range 8 to 20 mg per 100 ml urine(Oser, 1973). The urinary protein ranged from 15 to 600 mg with a mean value of 150.08± in urine was increased in patients over control values and this increase was statistically significant. ($P < 0.01$).
The mean values of serum urea and creatinine were increased over the control values and it was found to be statistically significant ($P < 0.01$) and the mean values of urine creatinine and urea were decreased than those of the control value and this decrease was found to be statisfically significant. ($P < 0.01$) Serum protein in the patients was decreased than the

the controls and urinary proteins were found to be elevated than normal and this increase was found to be statistically significant ($P < 0.01$). The increased excretion of urinary protein might to be due to some degree of kidney damage which could have been the result of urinary tract infection.

Table IV gives the urinary calcium and phosphorus levels in patients with urinary tract infection and controls and the individual values, are given in the Appendices X and XI respectively.

TABLE IV

URINARY CALCIUM AND PHOSPHORUS LEVELS IN PATIENTS WITH URINARY TRACT INFECTION AND CONTROLS.

Sample	Number	Urine Calcium		Urine phosphorus	
		Mg/100ml Mean ±SD	mg/100ml Range	mg/100ml Mean±SD	mg/100ml Range
Patients	34	10.44± 1.81	8.0 - 14.0	58.61± 6.50	48-75
Control	31	9.6± 1.17	8.0-11.5	53.13± 4.50	47-63

The mean value of urinary calcium in patients was found to be 10.44± 1.81 mg per 100 ml urine with a range of 8 to 14 mg per 100ml urine whereas in controls it ranged from 8.0 to

11.5 mg per 100ml with a mean value of 9.6± 1.17 mg per 100ml of urine. The mean value of urinary calcium in controls and in patients were within the normal range 7.5 to 13 mg per 100 g urin as reported by Harper (1977).

The mean value of urinary phosphorus in patients ranged from 48 to 75 mg per 100 ml with a mean of 58.61± 6.50 mg per 100 ml urine and the same in controls ranged from 47 to 63 mg per 100 ml urine with a mean of 53.13± 4.50 mg per 100 ml. The mean values of urinary phosphorus in patients and controls were within the normal range that is 45 to 70 mg per 100 ml urine (Harper, 1977). This suggested that the urinary calcium and phosphorus levels are not affected in urinary tract infection. Out of thirty six patients two patients alone excreted 120 mg of phosphorus and 16 mg of calcium per 100 ml urine. Hence these values were not included.

Table V gives the urinary haemoglobin levels in patients with urinary tract infection, and the individual values of urinary haemoglobin in patients are given in Appendix X.

TABLE V

URINARY HAEMOGLOBIN LEVELS IN PATIENTS WITH URINARY TRACT INFECTION

Number of patients	Urine haemoglobin	
	mg/100 ml Mean+SD	mg/100 ml Range
15	4.16 ± 2.99	1.1 - 12.4

The urine samples were first analysed for the presence of blood and then a quantitative estimation was made in those samples which showed the presence of blood. Fifteen of the patients exhibited haematuria and the haemoglobin values ranged from 1.1 to 12.4 g per 100 ml urine with a mean of 4.16 ± 2.99 g per 100 ml urine. This suggested that the infective organisms might have some how damaged the kidney function and lead to haematuria.

Table VI presents urinary sodium, potassium and chloride levels in patients with urinary tract infection and controls. Figure III Individual values of urinary sodium, potassium and chloride levels in patients and control are given in the Appendices X and XI respectively.

TABLE VI

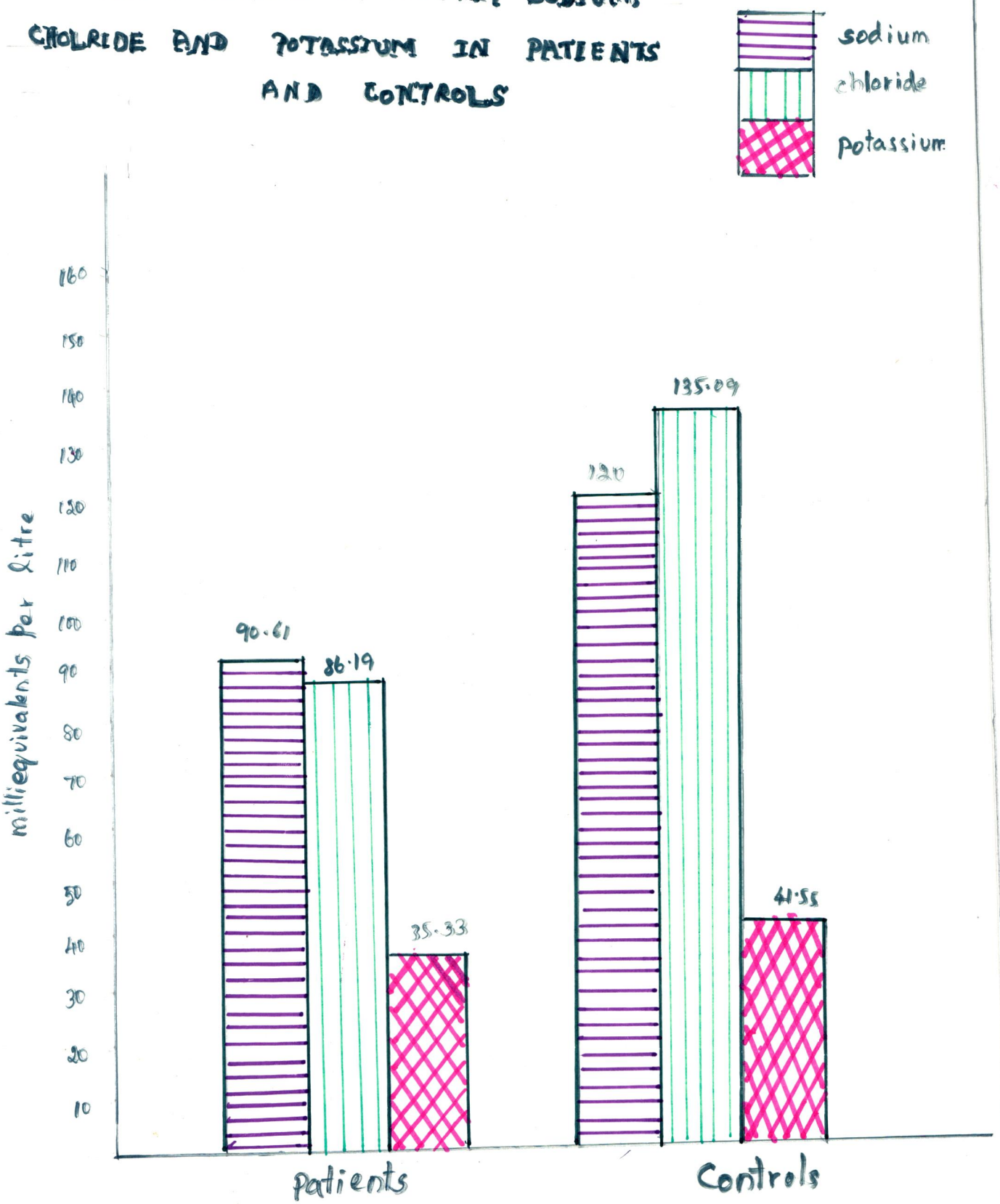
URINARY SODIUM, POTASSIUM AND CHLORIDE LEVELS IN PATIENTS WITH URINARY TRACT INFECTION AND CONTROLS:

Sample	Number	Urinary sodium		Urinary potassium		Urinary chloride	
		mEq/l Mean±SD	mEq/l Range	mEq/l Mean±SD	mEq/l Range	mEq/l Mean±SD	mEq/l Range
Patients	36	90.61± 19.56	60-120	35.33± 5.94	30-44	86.19± 29.16	38-142
Control	31	120±9.60	100-160	41.55±3.69	38-46	135.09±15.87	100-168
t value		7.969**		5.221**		8.679**	

** ($P < 0.01$)

Figure - 3

MEAN LEVELS OF URINARY SODIUM, CHLORIDE AND POTASSIUM IN PATIENTS AND CONTROLS



The mean value of urinary excretion of sodium in patients was found to be 90.61± 19.56 milliequivalents per litre of urine with a range of 60 to 120 milliequivalents per litre whereas in normal the mean level of urinary excretion of sodium was 120±9.60 milli equivalents per litre of urine with a range of 100 to 160 milli equivalents per litre. The urinary excretion of sodium in controls was within the normal range 100 to 150 milliequivalents per litre (Varley 1979). In patients the urinary excretion of sodium was decreased over the control values and this decrease was found to be statistically significant ($P<0.01$)

The urinary excretion of potassium in patients ranged between 30 to 44 milliequivalents per litre with a mean value of 35.33± 5.94 milliequivalents per litre of urine whereas in controls the values ranged from 38 to 46 milliequivalents per litre with the mean value 41.55± 3.69. The values of controls were within the normal range 38 to 45 milliequivalents per litre of urine (Varley, 1979). The mean value, of urinary potassium was decreased than the normal and this decrease was statistically significant ($P<0.01$)

The chloride excreted by the patients of urinary tract infection ranged from 38 to 142 milliequivalents per litre with a mean value of 86.19± 29.16 milliequivalents per litre of urine, whereas the excretion of chloride in controls ranged from 100 to 168 milliequivalents per litre with a

mean of 135.09± 15.87 milliequivalents per litre of urine. The control values were within the normal range given by varley (1979) which is 96 to 156 milliequivalents per litre of urine. The urinary chloride levels were found to be decreased in patients over the control and this decrease was statistically significant ($P < 0.01$).

This suggested that kidney function might have been damaged following urinary tract infection that the ability to conserve sodium, potassium and chloride could have been lost.

Table VII gives the mean pH values of urine in patients with urinary tract infection. The individual values of pH of urine in patients and controls are given in the Appendices X and XI respectively.

TABLE VII

MEAN pH VALUES OF URINE IN PATIENTS WITH URINARY TRACT INFECTION AND CONTROLS:

Sample	Number	Urine pH		t value
		Mean ±SD	Range	
Patients	36	6.89± 1.02	6.0-9.0	5.074**
Control	31	6.00± 0.24	5.6-6.5	

(P<0.01)

The mean pH value or urine in patients with urinary tract infection was found to be 6.89± 1.02 with a range of 6.0 to 9.0 whereas in controls it ranged from 5.6 to 6.5 with a mean value of 6.0 ± 0.24 In controls the pH value were found to be within the normal range 5.5 to 6.5 (Varley et al., 1980) In patients the pH values of urine were found to be increased and this increment was statistically significant, ($P < 0.01$) This is in confirmity with the finding of Parton (1960) who had reported that the increased pH of urine is patients was due to the urea splitting organisma present in urine.

Serum albumin globulin ratio of patients withurinary tract infection and controls are given in Table VIII. The individual values of albumin and globulin are given in the Appendices X and XI respectively.

TABLE VIII

SERUM ALBUMIN GLOBULIN RATIO OF PATIENTS WITH URINARY TRACT INFECTION AND CONTROLS:

Sample	Number	Albumin		Globulin	
		g/100ml Mean±SD	g/100ml Range	g/100ml Mean±SD	g/100ml Range
Patients	36	3.45± 0.47	3.2-4.4	2.30± 0.20	1.8-3.0
Controls	31	5.26± 1.36	4.0-6.0	2.67± 0.21	2.0-3.2
t value		1.200 **		NS 0.26	

** - ($P < 0.01$)
N.S - Not significant

The mean value of serum albumin in patients was found to be 3.405 ± 0.47g per 100 ml with a range of 2.8 to 4.4g per 100 ml whereas the value in controls was 5.46 ± 1.36g per 100 ml with a range of 4.0 to 6.0 per 100 ml. The control value were within the normal range 3.7 to 5.3g per 100 ml. (Varley et al., 1980). The mean value of serum albumin in patients was found to be increased over the control values and this increment was statistically significant ($P < 0.01$)

Serum globulin value in patients ranged from 1.6 to 3.0g per ml 3.0 g per ml with a mean value 2.09 ± 0.39 g per 100 ml whereas in controls the value ranged from 2.0 to 3.2g per 100 ml with a mean value of 2.67 ± 0.21 g per 100 ml. The mean serum globulin level in patients and controls were within the normal range 1.9 to 3.6 g per 100 ml (Varley et al., 1980) and no significant difference in globulin levels in patients and controls was noticed.

The albumin globulin ratio patients of urinary tract infection was found to be 1.5:1 but in controls it was 2:1. The change in the albumin-globulin ratio of patients was due to the decreased serum albumin level. This decrease in serum albumin level might be due to the increased excretion of this protein during infection.

Serum urea creatinine and protein levels in patients infected with E.Coli and in patients infected with other microbes are shown in Table IX.

TABLE IX

SERUM UREA, CREATININE AND PROTEIN LEVELS IN PATIENTS INFECTED WITH E.COLI AND IN PATIENTS INFECTED WITH OTHER MICROBES

Infecting organisms	Number of patients	Serum urea		Serum creatinine		Serum protein	
		Mg/100ml Mean±SD	mg/100ml Range	mg/100ml Mean±SD	mg/100ml Range	gl/100ml Mean±SD	g/100ml Range
E.COLI	22	70.23 37.95	24-168	3.0± 1.28	1.9-6.0	6.03± 0.65	4.5-7.0
Other microbes	14	75.93± 53.51	24-209	2.79± 1.34	1.9-6.0	5.91± 0.64	4.4-7.2
t value		NS 0.356		NS 0.476		NS 0.557	

N.S. not significant.

The mean value of serum urea was found to be 70.23±37.95 mg per 100 ml with a range of 24 to 168 mg per 100ml in patients infected with E.Coli and in other microbial infection the mean was 75.93± 53.51 mg per 100ml with a range 24 to 209 mg per 100 ml. There was no significant change in serum urea level affected by the action of E.Coli and other microbes

The serum creatinine level in both groups of patients ranged from 1.9 to 6.0 mg per 100 ml with a mean value of 3.0 ± 1.28 mg per 100 ml in the case of those infected with E.Coli and 2.79± 1.34 mg per 100 ml in patients infected

with other microbes. No significant difference in serum crea inine levels was noticed between the two groups

The mean value of serum protein in patients infected with E.Coli was found to be 6.03± 0.65g per 100 ml with a range 4.5 to 7.0g per 100 ml and in patients infected with other microbes the values ranged from 4.4 to 7.2 g per 100 ml with a mean of 5.91± 0.64 g per 100ml. However no significant difference between the serum protein levels of the two groups of patients was noticed.

Table X gives urinary urea, creatinine and protein levels in patients infected with E.Coli and in patients infected with other microbes,

TABLE X

URINARY UREA, CREATININE AND PROTEIN LEVELS IN PATIENTS INFECTED WITH E.COLI AND IN PATIETS INFECTED WITH OTHER MICROBES.

Infective organisms	Number of patinets	Urine urea		Urine creatinine		Urine protein	
		g/100ml Mean±SD	g/100ml Range	mg/100ml Mean±SD	Mg/100ml Range	mg/100ml Mean±SD	mg/100ml Range
E.Coli	22	0.93± 0.259	0.589-	48.5± 16.67	30-85	147.13± 169.5	15-600
Other microbes	14	0.99± 0.27	0.503- 1.39	52.73± 16.63	35.94	177.73± 184.41	15-500
t value		0.683 NS		0.759 NS		0.512 NS	

N.S. not significant

The mean urinary urea excretion inpatients with E.Coli infection was found to be 0.93 ± 0.25 g per. 100 ml Urine

100ml urine with a range of 0.589 to 1.32 g per 100 ml whereas in patients infected with other microbes the urine urea level was ranged from 0.503 to 1.390 g per 100 ml with a mean of 0.99± 0.27 g per 100 ml of urine. No significant difference between the urinary urea levels of the two groups of patients was noticed.

The urinary creatinine excreted inppatients with E.Coli infection was found to be 48.50± 16.67 mg per 100ml of urine with a range of 30 to 85 mg per 100ml urine whereas in patients with other microbial infection the urinary creatinine ranged from 35 to 94 mg per 100ml with a mean of 52.73± 16.63 mg per 100 ml of urine. There was no significant difference in the vales of creatinine excreted by the patiets infected with E.Coli and those infected with other microbes.

The urinary protein values ranged from 15 to 600 mg per 100 ml with a mean of 147.13± 169.5 mg per 100ml of urine in patients infected with E.Coli and in patients infected with other microbes the value ranged from 15 tp 500 mg per 100 ml with a mean of 177.73± 184.41 mg per 100ml of urine. No significant difference in the urinary excretion of protein by the patients infected with E.Coli and those infected with other microbes was noticed.

Urinary sodium, potassium and chloride levels in patients infected with E.Coli and in patients infected with othermicrobes are shown in Table XI.

TABLE XI

URINARY SODIUM, POTASSIUM AND CHLORIDE LEVELS IN PATIENTS INFECTED WITH E.COLI AND IN PATIENTSINFECTED WITH OTHER MICROBES.

Infective organism	Number of patients	Urine sodium		Urine potassium		Urine chloride	
		mEq/l Mean±SD	mEq/l Range	mEq/l Mean±SD	mEq/l Range	mEq/l Mean±SD	mEq/l Range
E.Coli	22	95.73± 20.72	60-134	35.73± 4.23	30-42	92.14± 29.70	50-142
Other microbes	14	84.80± 16.11	60-122	35.23± 4.31	30-44	80-07± 26.91	38-142
		1.801 NS		0.370 NS		1.284 NS	

N.S. Not significant.

The urinary sodium levels ranged from 60 to 134 milli-equivalents per litre with a mean of 95.73± 20.72 milli-equivalents perlitre urine ~~of~~ in patiets infected with E.Coli and in patients infected with other microbes the value ranged from 60 to 122 milliequivalents per litre of urine with a mean of 84.80±16.11 milliequivalents per litre. There was no significant difference between the level or urinary sodium excretion of the two groups of patients.

The urinary potassium levels ranged from 30 to 42 milliequivalents per litre of urine with a mean of 35.73± 4.23 mEqper litre of urine in patients infected with E.Coli and in patients infected with other microbes the meanlevel of urinary potassium was 35.23±4.31 milliequivalents per litre urine with range of 30.00-44 milliequivalents per litre urine. There was no significant difference between the urinary potassium levels ofthe two groups of patients was noticed.

The mean level of chloride in patients infected with E.Coli was 92.14± 29.70 milliequivalents per litre urine with a range of 50 to 142 milliequivalents per litre. In patients infected with other microbes the mean urinary chloride level was 80.07± 26.91 milliequivalents per litre urine with a range 38 to 142 milli equivalets per litre urine. It shows that there was no significant difference between the urinary chloride levels of the two groups of patients.

This indicated that there was no difference in the effect of E.Coli and other microbes in the urinary tract infection with regard to the excretion of sodium, potassium and chloride.

The mean pH values of urine in patients infected with E.Coli ad in patients infected with other microbes are shown in table XII.

TABLE XII

MEAN pH VALUES OF URINE IN PATIENTS INFECTED WITH E.COLI AND IN PATIENTS INFECTED WITH OTHER MICROBES.

Infective organisms	Number of patients	Urine pH	
		Mean ±SD	Range
E.Coli	22	6.18± 0.28	5.5 - 6.2
Other microbes	14	7.89± 0.84	6.5 - 9.0
t value		7.603**	

** (P< 0.01)

The mean pH value of urine in patients infected with E.Coli was found to be 6.18± 0.28 with a range of 5.5 to 6.2 whereas in patients infected with other microbes the mean value of pH of urine was 7.89± 0.84 with a range of 6.5 to 9.0. The pH of the urine in E.Coli infection was acidic and in patients infected with other microbes the pH of the urine was alkaline. The difference in the mean pH values of urine patients infected with E.Coli and of those infected with other microbes was found to be statistically (P 0.01) significant. This suggested that the increased urinary pH values of patients infected with other microbes might be due to the break down of urea

SUMMARY AND CONCLUSION

V. SUMMARY AND CONCLUSION

This investigation was undertaken to study the biochemical changes in urinary tract infections. Thirty six patients were selected for this study. The age of the patients ranged between 15 and 85 years. Of the thirty six patients eleven were females and the rest males.

Blood and urine samples were collected from these patients. Serum and urinary urea, creatinine and protein, urinary calcium, phosphorus haemoglobin, sodium, potassium and chloride and pH were determined in all of them and in thirty one normal healthy volunteers of same age, and sex who were also free of diseases. The conclusions arising out of this study are:

1. Out of the thirty six patients suffering from Urinary tract infection twenty two were infected with E.Coli; seven patients had Klebsiela infection; four patients had Pseudomoas aeruginosa infection; 3 patients had Proteus vulgaris infection; It was found that infection by E.Coli was more prevalent. Sixty one per cent of the patients had E.Coli infection
2. Serum urea and creatinine levels in patients were found to be significantly increased ($P < 0.01$) over the control values. The mean serum urea creatinine

levels in patients was 72.81 ± 48.38 mg per 100 ml and2.93 ± 1.32 mg per 100 ml respectively. In controls the mean surum urea and creatinine leves were found to be 33.16± 4.72 mg per 100 ml and 1.85 ± 0.14 mg per 100 ml respectively. The increased serum urea and creatinine during infection might have been due to the kidney failure.

3. The mean value of serum protein in patience was significantly ($p < 0.01$) increased than the control value. The mean value of serum protein in patients was found tobe 6.04 ± g per 100 ml and in control it was 8.14 ± 0.51g per 100 ml. This decreased serum protein may be due to the increased excretion of protein in urine during infection.

4. The mean values of urea and creatinine excreated in patients were found to be significantly ($p < 0.01$) decreased than the control values. The mean values of urinary urea and creatinine were 0.93 ± 0.30g per 100 ml of urine and 50.28 ± 17.01 mg per 100 ml and of urine respectively and in controls the same were 1.39 ± 0.11 g per 100 ml and 71.06 ± 10.52 mg per 100 ml of urine. This decreased values of urinary urea and creatinine might be due to thekidney failure causing increased serum urea and creatinine.

5. The mean value of protein excreated in urine in patients was found to be significantly increased ($p < 0.01$) over the control value. The mean value of urinary protein in patients was found to be 150.08 ± 162.26 mg per 100 ml urine and in controls it was 15.55 ± 4.14 mg per 100 mg of urine. This increased excretion of protein might be due to the inability of the kidney to retain the protein.

6. No significant difference was seen in the urinary calcium ad phosphours levels between the controls and the patients. The mean values of urinary excretion of calcium and phosphours in patients were found to be 10.44 ± 1.81 mg per 100 ml and 58.61 ± 6.50 mg per 100 ml of urine respectively The mean values of urinary excretion of calcium and phosphorus in controls were 9.6 ± 1.17 mg per 100 ml and 53.13 ± ~~mg per~~ 4.50 mg per 100 ml of urine respectively. During urinary tract infection the urinary excretion of calcium and phosphorus is unaffected.

7. The mean value of urinary excretion of soldium in patients was found to be significantly ($p < 0.01$) decreased over the controls. The mean value of sodium excreted in urine of patients was foud to be 90.61 ± 19.56 milli equivalent per litre and in centrols it was 120.± 9.60 milli equivalent per litre. This decreased uninary excretion of sodium might be due to the damage of kidney as the result of the infection.

8. The mean value of urinary excretion of potassium in patients was found to be significantly (p.0.01) decreased than the control values. The mean value of potassium excreted in urine of patients was 35.33 ± milliequivalents per litre and in controls it was 41.55 ± 3.69 milliequivalents per litre of urine. This decreased excretion of potassium in urine might be due to the inability of the kidney to excrete excessive potassium.

9. The urinary excretion of chloride level in patients was found to be significantly (p 0.01) decreased over the control values. The mean value of urinary chloride in patients was found to be 86.19 ± 29.16 milliequivalents per litre and in controls it was 135.09 ± 15.87 milliequivalents per litre of urine. This decreased levels of urinary chloride in patients might be due to the in ability of kidney to conserve chloride.

10. The pH value of urine in patients was significantly (p 0.01) increased over the control values. The mean pH value of urine in patients with urinary tract infection was found to be 6.89 ± 1.02 and in controls it was 6.0 ± 0.24 This was suggested that the increased urinary pH in patients with urinary tract infection might be due to the presence of urea splitting organisms in urine.

11. The serum albumin globulin ratio was found to be decreased in patients over control. The mean albumin and globulin values of patients were 3.17 ± 1.64 g per 100 ml and 2.09 ± 0.39g per 100 ml respectively. The mean albumin,

globulin values of controls were found to be 5.26 ± 1.36g per 100 ml and 2.67 ± 0.21 g per 100 ml respectively. The mean albumin globulin ratio in controls was found to be 2:1 whereas in patients it was 1:5. This might be due to the decrease in the serum albumin level and this decrease in the serum albumin level and this decrease in the serum albumin level might be due to the increased excretion of this protein during infection.

12. The serum and urinary urea, creatinine, protein levels in patients infected with E.coli and patients infected with other microbes showed no significant difference in these biochemical parameters which indicates that the effect of E.coli and the other microbes were similar.

13. The mean values of urinary sodium, potassium and chloride in patients infected with E.coli and in patients infected with other microbes showed no significant difference. This indicates that the effect of E.coli and other microbes on these biochemical parameters were similar.

14. The pH value of the urine in patients infected with E.coli were statistically increased (p 0.01) over the patients infected with other microbes. The mean pH value of the urine in patients infected with E.coli was 6.18 ± 0.28 and the mean pH value of the urine in patients infected with other microbes was found to be 7.89 ± 0.84. This increased pH of the urine was due to the break down of urea

by other microbes. In E.coli infection, the urine was acidic and in other microbial infection the urine was alkaline in nature.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Agarwal, G.R. 1982 'Drip infusion pyelography in-chronic renal failure'. Ind. Med. Gaz CXVI, 4: 125-129.

2. Ananthanaryan, C.K., Jayaram panicker. 1975 'Text book of microbilogy'. Second edition, orient Longman. PP.257, 258-265.

3. Antiam, F.P. 1975 'Clinical diabetics and nutrition' II nd edition, Oxford medical publication PP 497-500

4. Bhupendera M. Tolia, Alfredo I. Loreta, Selwyn Z. Freed, Bernard Fruchtman. 1981. 'Xanthogranulomatous pyelonephritis' J. Urol, 126:437-439.

5. Campbell.P.M., Harrison 1970 'urinary infections'- Urology' W.B. Saunders Company.Vol.2 PP 1974-1983.

6. Chakraborty.P., Metha, M.J., Roy, P.B., Mrs. Shah., A.D., Roy's. 1972 Urinary tract infection' J. Ind. Med. Asso, 58:113.

7. Dodson, A.T. 1970 'Surgical treatment of renal infections. Fourth edition, the C.V. Mosby Company PP-150-156.

8. Doherty, N. Grady, F.O., Brumfitt, W. 1968 'Urinary tract infections' J. Ind. Med. Asso. 65:57.

9. Doifode, S.M. Deshpande, A.M. Patil, A.W. 1982 'Comparitive study of the drugs used in urinary tract infection'. Ind. Med. Gaz, CXVI, 6 : 167-168.

10. Engel, G. Antheny, J. Schaeffer, John T. Gray back and Ear F. Wendel, 1980 The role of excretory urography and cystoscopy in the evaluation and management of women with recurrent UII' J.Urol,123:184-186

11. Ghalaut, P.S. Prakash, C.P. 1982 'Incidence of asymptomatic UTI in Indian General Population. A community survey. Ind. Med. Gaz, CXVI:7, 221-223.

12. Gulati, P.D., Rakesh Bharti and Rixvi 1981 'Evaluation of double strength Trimethoprim sulpha Methaxazole (nicotnixmx m-s) in Urinary and respiratory tract infections Ind.Med CXVx Gaz, CXV:9, 308-310.

13. Harrison Giter, Perlmutter. Stamly., walsh. 197 1979 'Management of renal and urinary tract disorders during pregnancy'. 'Campebell's urology'. W.B. Saunders Company. Vol. 3. PP-642-643-

14. Harold Harper, Victor W. Rodwell and peter Mayes R. 1977 'Review of Physiological Chemistry" 17 th edition, Maruzen Asian edition, PP-642-643

15. Kamat R.V. Kelkar S.S. 1980 'Serum agglutinins to infecting organisms in cases of 'UTI' Ind. Med. Gaz, CXV:9,342-344.

16. Kass E.H., Kunin, C.M., Beard, R.W., Roberts,A.P., 1956 'Urinary tract infection' Trans. Ass. Amer. Physicians, 69-56.

17. Kinacid Smith
1965
'Bacteriuria in pregnancy' J. obstet, Gynaecol, 7 : 728-729

18. Krieger J.N., Ellen Leary-Zombek, Andrea Scheidt and lewis M. Drusin,
1980
'A nosocomial epidemic of antibiotic resistant Serratia marcescens Urinary tract infections' J.Urol.,124: 498-501

19. Mc. Fayden, I.R., Eykyn. S.J. Gardner N.H., Vanier T.M., Bennett A.E., Mayo M.E., and Lloyd Davies R.W.
1973
'Bacteriuria in pregnancy' J. Obstet, Gynaecol, Brit Commonwealth, 80:385

20. Martin D.W., Mayes, P.A., Rodwell,V.W.,
1981
'Harper's review of biochemistry 18th edition. Maruzen Asian edition. PP 551-556.

21. Mukerjee, A.B.,
1976
'Urinary tract infection' J.Ind. Med. Asso.58:179

22. Mathur M., Bhadury, S, Kumar,A.
1981
'Multiple urolithiasis with a solitary renal calculus'-A case report Ind. Med. Gaz., 12, 431-432.

23. NIN.
1971
'A manual of laboratory techniques' Indian institute of nutrition P-125.

24. Oser, B.L., 1976 — 'Hawk's physiological Chemistry' Tata Mc Graw-Hill publishing company Ltd., Bombay., PP-1016-1045

25. Osofsky, H.J., Geolge Schalfer, 1978 — 'Urinary tract infections in pregnancy' clin obstet. gynaeclol, Harper and Raw publishers, London, PP-921-928

26. Parsons, L. Joseph and Schmidt. 1981 — IUrinary tract in pregnancy' J.Urol. 125:452

27. Parton, I. 1960 — 'Urinary infections' Urology in general practice', Butter worths London. P-49

28. Patnaik, S.R., Padhy, P.K., Panda., P.L. Patnaik, B.C., Panda, R.K. Moharana S.N. Misra S.C., 1983 — 'Clinicobacteriological study of UTI in southern orissa with special feference to drug sensitivity Ind. Med. Gaz., CXVII :2, 42-46

29. Purwar, V.N., Agarwal, S.P., Dikshit, S.K., 1972 — 'Gram stained urine slides in the diagnosis of urinary tract infection in children J. Ind. Med. Asso. 11: 387-389

30. Roberts, T. 1983 — 'Urinary tract infection' J. Urol 128: 1102-1104

31. Roderick D. Turner, Donald A. Charnock 1963 'Urinary tract infections' 'Office Urology' Mc. Grawhill book company inc. PP-41-45

32. Ronald P. 1981 'Blood group and urinary tract infection'. The Lanet 25:4, 946

33. Singh, U.K., Bela Saigal 1981 'UTI' Bacterial flora and antibiogram at Gorakpur Ind. Med. Gaz., CXV. 8, 267-270

34. Smith H.W. 1970 'The kidney structure and function in health and disease'. Oxford University press, PP-63-65.

35. Smith D. 1978 'Urinary tract infections' General urology Ninth edition, Lange Medical Publication, PP-145-203

36. Sussman M.L., Geolge Jacobson, Howard Jayne,E. 1967 'Urinary tract infections' Urologic Roentgenology, Raw Publishers London.PP-8-120

37. Svanborg Eden.C., Gunilla Lidin Janson and Lindberg 1979 'Adhesiveness to urinary tract epithelial cells of fecal and urinary E.coli isolates from patient with symptomatic UTI of Asymptomatic bacteriuria of Varying duration'. J.Urol, 122:185

38. Tietz.N. 1976 'Fundamentals of clinical chemistry'W.B. Saunders Company London PP 980-982

39. Tomlinson, J.L., Cole J.G., and Gope 1969 'Urinary tract infection' E.Brit. Med. J, 1:29-

40. Turner.T. 1961 'Urinary tract infection' the Lancet, 2:1062

41. Upadhyay, P.K., Srivastava, R.G., Singh, Y.N., Singh V.P., Usha, K. Tripathi 1980 'A study of anaemia in chronic renal failure' Ind. Med. Gaz., CXV. 7, 252-256

42. Varley, H., Alan H.Gowenlock, Maurice Bell 1980 'Practical Clinical Biochemistry Fifth edition, Vol. I. Willam Heinemann Medical books Limited, PP 597,607,459,460,463,486,776.

43. Varma N.C., Taneja O.P., Sasiena S.N., 1972 (Recurrent urinary tract infection in females' J.Indi.Med. Asso. 58:5, 171.

44. Vinod Kumar S. Gupta K. 1982 'Staphylococcus Saprophyticus in urinary tract infection'. Ind. Med. Gaz, CXVI:9,270.

45. Warren J.W., Herbert L. Muncie, Erick, J.R., Bergquist, J., Johm, M. Hoopes 1981

'Sequelae and managements of Urinary infection in the patient requiring chronic catheterization' J. Urol, 126:10, 437-439

46. White A.,Handler, P., Smith E.L., Robert L. H Hilli, Robert Lehman, I. 1978

'Principles of Biochemistry' Sixth edition, International student edition.
PP. 1076-1080.

APPENDICES

VII APPENDICES

APPENDIX I

ESTIMATION OF CREATININE

ALKALINE PICRATE METHOD

(Varley et al., 1980)

PRINCIPLE:

Creatinine when treated with an alkaline picrate solution forms a red coloured complex. This is known as Jaffe's reaction. The intensity of the colour developed is compared in the colorimeter at 540 millimicron.

REAGENTS:

1. 0.04 M picric acid
2. 0.75 N sodium hydroxide
3. Stock standard solution:

100 mg of creatinine was dissolved in N/10 hydrochloric and made upto 100 ml with the same.

4. Working standard:

2.0 ml of the stock solution was diluted to 100 ml with water.

PROCEDURE:

Took 3.0 ml of water, added 2.0 ml of serum, 1.0 ml of 10% sodium tungstate solution and 2.0 ml of 2/3 N

solphuric acid. Kept for 10 minutes and centrifuged. 3.0 ml of the supernatant was pipetted out in a test tube. 1.0 ml of urine was diluted to 100 ml and 3.0 ml this was taken for the experiment. Tubes containing 0.5 - 2.5 ml of the working standard solution were taken and the volumes of these tubes were made upto 3.0 ml with water. Alongwith these a blank was also prepared. Added to all the tubes 1.0 ml of 0.04M picric acid and 1.0 ml of 0.75 N sodium hydroxide and let it stand for 20 minutes for the colour to develop. Shook well and the colour developed was compared with the standard against reagent blank at 540 millimicron.

A standard graph was drawn by takingthe concentration of creatinine on the X-axis and colorimeter reading on Y - axis. From this the concentration of creatinine in serum and urine was calculated.

APPENDIX II

ESTIMATION OF UREA

ii) DIACETYL MONOXIME THIOSEMICARBAZIDE METHOD

(Varley et al., 1980)

PRINCIPLE:

Urea directly reacts with diacetyl monoxime in the presence of thiosemicarbazide to form a red coloured product which is measured colorimetrically at 540 millimicron.

REAGENTS

1. Trichloro acetic acid 100g/litre
2. Stock Diacetyl monoxime 2.5g/100 ml water
3. Stock this semicarbazide 0.25 g/100 ml water
4. Acid ferric chloride

Added 1 ml sulphuric acid to 100 ml of a ferric chloride solution containing 50g/litre in water.

5. Acid reagent:

Water 100 ml

Concentrated sulphuric acid 8 ml

Phosphoric acid 20 ml

5 Per cent ferrichloride 1.0 ml

6. Colour reagent:

Acid reagent 30 ml

Water 20 ml

2.5 per cent diacetylmonoxime 1.0 ml

0.25 per cent Thiosemicarbazide 0.25 ml

Colour reagent should be prepared just before use since the solution is not stable for more than an hour.

7. Stock standard:

100 mg of urea/100 ml water

Dissolved 100 mg of urea in distilled water and made up to 100 ml with the same.

8. Working standard:

2.0 ml of the stock standard was diluted to 100ml with water. 1.0 ml of this solution contains 20 microgram of urea.

PROCEDURE:

Added 1.0 ml water and 1 ml of Trichloro acetic acid to 0.2 ml serum. Mixed well. ~~Centrifuged~~ Centrifuged. Took 0.2 ml supernatat for the experiment. 1.0 ml of the urine was diluted to 100 ml with water in a standard flask, Pipetted out 0.4 ml of this solution into a test tube.

Into a series of test tubes took 0.5 - 2.5 ml of standard urea solution, the microgram values correspond

to 10-50 microgram. The volume of all the tube was made upto 3.0 ml with water. Added 5.0 ml of the colour reagent. Mixed well. Corked and heated in a vigorously boiling water bath for 20 munutes. Along with this a blank was also conducted. Removed the tubes and cooled. Readings were taken against a reagent blank at 540 millimicron.

A standard graph was drawn by taking concentration of urea on X axis and colorimeter readings on Y axis. From this the amount of urea present in serum and ~~ureine~~ urine was calculated.

APPENDIX III

ESTIMATION OF TOTAL PROTEIN AND GLOBULIN RATIO

BIURET METHOD

(Varley et al., 1980)

PRINCIPLE

The colorimetric method for protein estimation makes use of Biuret's reaction. Substances which contain $CONH_2$ groups joined directly or through a single carbon or nitrogen atom give a blue purple colour, with alkaline copper sulphate solution. Proteins thus give a purple colour which is different for different proteins. The reaction takes its name from the complex formed that is 'Biuret'.

REAGENTS:

1. Stock Biuret reagent:

Dissolved 45g of Rochelle salt in about 400 ml of 0.2 N sodium hydroxide and 15 g of copper Sulphate (Cuco4.5H2o) stirring continuously until the solution was complete. Added 5.0 g of potassium iodide and made upto a litre with 0.2 N sodium hydroxide.

2. Dilute Biuret reagent:

Diluted 200 ml of stock reagent to a litre with 0.2N sodiumhydroxide which contains 5 g of potassium iodide per litre.

3. Standard protein solution:

Weighed 400 mg of albumin and dissolved in 0.9% saline solution and made upto 100 ml with the same so that 1.0 ml of this solution contains 4 mg of protein.

4. 0.9 per cent Saline

5. 22.5 per cent sodium sulphate solution,

PROCEDURE:

Into a series of test tubes added 0.5 - 2.5 ml of standard protein solution. The volume was made upto 2.5 ml with water. Into another tube pipetted out 0.4 ml serum and diluted with 0.9 per cent saline to 10 ml. From this 2.5 ml of solution was taken for the experiment. Now added 3.0 ml of diluted Biuret reagent to all the tubes. Along with this a blank was taken. The colour developed was read at 500 millimicron colorimetrically after 30 minutes. A standard graph was drawn by plotting concentration on x axis and Klett radings on Y axis. The amount of protein present was calculated. This gives total protein value of serum.

PRECIPITATION OF GLOBULIN:

Globulin was precipitated by mixing 0.4 ml of serum with 9.6 ml of 22.5 per cent sodium sulphate. Stoppered the tubes and leftin the incubator at 40°C over night. Filtered the solution next day using whatman number 42 filter paper. Took 2.5 ml of filtrate and carried out the experiment as for the rest. The concentration of gram percentage of albumin present in globulin free filtrate was detected from the graph.

APPENDIX IV

ESTIMATION OF PHOSPHORUS

(Fiske and Subbarow Method)

(Oser, 1976)

PRINCIPLE

Phosphate reacts with molybodic acid to form phosphomolybdic acid. This on treatment with 1,2,4 aminonaphtholsulphanic acid, is selectively reduced to produce a deep blue colour which is probably due to the mixture of lower oxides of molybdenum. This colour is compared at 660 millimicron in a colorimeter with that obtained from a standard phosphate solution treated in the same way.

REAGENTS:

1. 10N Sulphauric acid
2. Molybdate Isolution:

   2.5 per cent of Ammonium Molybdate in 5 N sulphuric acid.

3. Molybdate II Solution:

   2.5 per cent Ammonium Molybdate in 3N sulphuric acid.

4. 1, 2, 4 Aminonaphthol sulphonic acid:

   Placed 195 ml of 15 per cent sodium bisulphate solution in a glass stoppered cylinder. Added 0.5g of

1,2,4 Aminonaphthal sulphonic acid. Added 5.0 ml of 20 per cent sodium sulphite solution. Stoppered and shook well until the powder dissolved. If the solution is not complete add more sodium sulphite 1.0 ml at a time with shaking but avoid in excess. Transferred to a brown bottle and stored in cold.

5. Stock standard Phosphate solution:

Accurately weighed 35.1 mg of pure monopotassium dihydrogen phosphate. 1.0 ml of 10N sulphuric acid was added and made upto 100 ml with water in a standard flask.

6. Working standard:

10.0 ml of the stock standard solution was diluted to 100 ml with water, so that 1.0 ml of the working standard contains 8 ~~micro~~milli micron phosphorus.

PROCEDURE:

Into a series of test tubes pipetted out 1.0, 2.0 3,0, 4.0 and 5.0 ml of the workig standard. Then 1.0 ml of the urine sample was diluted to 100 ml with water 1.0 ml of this was taken for the estimation. Added ammonium molybdate I solution to the stadard and 1.0 ml of Molybdate II solution to the urine samples. The The volume in all the tubes was made upto 9.6 ml with

water and then added 0.4 ml of Aminonaphthol sulphonic acid to all the tubes. Mixed well and allowed 20 minutes for the colour development. The blue colour developed was read in a colorimeter against a reagent blank at 660 millimicrons.

A Standard graph was drawn by taking concentration on x axis and colorimeter readings on yaxis. From this the concentration of phosphorus in urine sample was calculated

APPENDIX V

ESTIMATION OF CALCIUM

Method of Clark and Collip

(Oser, 1976)

PRINCIPLE:

Calcium is precipitated as oxalate directly from ~~dissolved~~ the urine and after washing the precipitate is dissolved in acid and titrated against permanaganate.

REAGENTS:

1. ~~4~~ per cent Ammonium Oxalate
2. 2 per cent Ammonia solution (V/V)
3. 0.01 N potassium permanganate.

Prepared freshly before use by diluting a stock solution of 0.1N potassium permanaganate.

4. Approximately normal sulphuric acid.

PROCEDURE:

To 2.0 ml of urine added 2.0 ml of water and 1.0 ml of 4% Ammonium oxalate and allowed to stand overnight. The precipitated calcium oxalate was centrifuged. The supernatant was removed without disturbing the precipitate. Added 3.0 ml of 2 per cent ammonia solution down the sides of the tube and mixed the precipitate well. Centrifuged and poured the supernatant. This was repeated 2-3 times until the wasted solution gave no pretipitate with calcium chloride solution. This was done to remove excess Ammonium oxalate. Added 2.0 ml of approximately normal sulphuric acid.

Mixed and dissolved the precipitate with the acid, warmed by placing in a beaker containing almost boiling water to complete the solution of oxalate. Removed and titrated with 0.01 N potassium permanganate keeping the mixture at 70-75°C to a faint pink colour which persisted for about one minute. As a blank titrated 2.0 ml of 1 N sulphuric acid to the same and point. The difference between the two titer values gives the volume of 0.01 N potassium permanganate required to titrate the calcium oxalate.

Calculation:

1.0 ml of 0.01N potassium permanganate is equivalent to 0.2 mg of calcium.

Milligram of calcium per 100ml = (Titration of urine - Blank) $\times \frac{0.2 \times 100}{2}$

APPENDIX VI

ESTIMATION OF CHLORIDE

Method of Van Slyke

(Varley, 1975)

PRINCIPLE:

Chloride is precipitated as silver chloride by the addition of silver nitrate solution. Excess of silver nitrate is titrated against standard thiocyanate in the presence of ferric alum as indicator.

REAGENTS:

1. 0.05N silver nitrate solution:

Dissolved 8.495g of silver nitrate in water and made up to a litre with water. The solution which should be standardised against sodium chloride was kept indefinitely in a brown bottle.

2. 0.02N Potassium thiocyanate:

Prepared at intervals by diluting a stock 0.1N solution.

3. Concentrated nitric acid

4. 5 per cent solution of ferric alum

PROCEDURE:

To 1.0 ml of the urine added 3.0 ml of silver nitrate solution and 2.0 ml of concentrated nitric acid. Heated over a flame till a pale yellow colour was obtained. Cooled and

and then added 6.0 ml of Ferric alum. Then titrated with 0.02N thiocyanate (Standardised against silvernitrate of known strength) until a raddish brown colour persisting for 10-15 seconds was obtained.

To determine the standard 2.0 ml ofconcentrated nitric acid, 3.0 ml of silver nitrate and 6.0 ml of ferric alum were taken and titrated against 0.02N thocyanate to the same end point as above.

CALCULATIONS:

The difference between the two titrations gives a measure of the amount of chloride in 1.0 ml of urine interms of 0.02 N solution.

M.Eq. of chloride/litre (Ml titration of standard - ml titration of the unknown)

APPENDIX VII

DETERMINATION OF URINARY PROTEIN (TECHNIQUE OF RICHTERICH)

(Varley et al., 1980)

REAGENTS:

1. Perchloric acid:

Diluted 5.7 ml of concentrated acid specific gravity 1.70 to 100 ml with water.

2. Biuret reagent as for the determination of serumprotein

3. Albumin standard.

Weighed 400 mg of Albumin and dissolved in 0.9% saline solution and made upto 100 ml with the same so that 1,0 ml of this solution contains 4 mg of protein.

PROCEDURE:

To 2.5 ml urine added 2.5 ml of ice cold perchloric acid Allowed to stand for 10 minutes. Centifuged vigorously. Disscarded the supernatant and added 3.0 ml of biuret reagent mixed well and read at 540 mm.

Into a series of tubes added 0.5 - 2.5 ml of standard protein solution. The volume was made upto 2.5 ml with water. Added 3.0 ml of diluted Biuret reagent to all the tubes. Alongwith this a blank was taken. Thecolour developed was read at 540 millmicron colorimetrically.

A standard graph was drawn by plotting concentration on X axis and klett readings on Y axis. The amount of protein present in the urine was claculated.

APPENDIX VIII

HAEMOGLOBIN ESTIMATION BY CYANMETHAEMOGLOBIN METHOD (NIN 1971)

REAGENTS:

Drabkin's Diluent solution:

1. Sodium Bicarbonate 1g
2. Potassium cyanide 0.05g
3. Potassium ferricyanide 0.20g.
4. Distilled water to make 1 litre.

This solution should not be used after it forms a precipitate at the bottom, of storage bottle. The solution preserved in a dash brown bottle & prefereably under cold storage. Its preparation and handling should be done with great care.

PROCEDURE:

1. Exactly 5.0 ml of Drabkins diluent solution was measured into a dry test tube from a burette or a pipette with suction bulb.
2. Exactly 1.0 ml of urine was tranferred from a standard haemoglobin pipette into the diluent solution. Usual care in filling and cleaning of loaded haemoglobin pipette must be observed.
3. The pipette was rinsed 3 times with the diluent solution without allowing the formation of air bubbles in the solution.
5. 10 minutes time was allowed for the formation of the cyanmethemoglobin.

5. The urine and dilute were the thoroughly mixed by rotating the tube.

6. 5 ml of the diluent solution was used as blank.

7. The readings were taken in a photoelectric colorimeter at 540 millimicron.

CALIBRATION PROCEDURE:

1. Total blood iron was determined by Wang's method. This determination would give absolute amount of Haemoglobin.

2. Exactly 0.02 ml of this known blood sample was measured as aboe into 5.0, 7.5, 10.0, 12.5 and 15.0 ml respectively of diluent solution and mixed by rotating the tubes. These solutions are now equivalent to blood samples containing respectively 100, 67, 50,40 and 30 per cent of the original haemoglobin concentrations.

3. The intensity of the colour was read using a green filter (540 millimicron) against a blank set at zero optical density.

A standard graph was drawn using these haemoglobin concentration and corresponding optical density value.

APPENDIX IX

ESTIMATION OF SODIUM AND POTASSIUM

Flame photometer

(Varley et al., 1980)

PRINCIPLE:

Atoms of many metallic elements when given sufficient energy such as that supplied by a hot flame, will emit this energy at wavelengths characteristic for the element. A specific amount of thermal energy is absorbed by an orbital electron. The excited electrons release their excess energy as photons of particular wavelength as they change from the existed to their ground state. Under controlled conditions the light intensity of the characteristic wavelength produced by each of the atom is directly preportional to the number of atoms that are emitting energy which is proportional to the concentration of the substance.

REAGENTS:

1. Standard potassium solution:

1.907 g potassium chloride was dissolved in distilled water and made upto 100 ml. 10.0 ml of the solution was diluted to 100 ml to form a solution containing 0.1 mg potassium per ml.

2. Standard sodium solution:

2.54 g sodium chloride was dissolved in distilled water and made upto 100 ml 10.9 ml of the solution was diluted to 100 ml to form a solution containing 0.1 mg sodium/ml.

PROCEDURE:

Different concentrations of standard potassium chloride was fed into flame photometer. The transmittance was recorded using potassium filter. Then the calibration curve was prepared. The sample was treated in the same manner as that of standard solutions and from the calibration curve, the amount of potassium was calculated.

The same procedure was repeated for the estimation of sodium using sodium filter.

APPENDIX X

SERUM AND URINARY UREA, CREATININE, PROTEIN AND URINARY pH, CALCIUM PHOSPHORUS, HAEMOGLOBIN SODIUM, POTASSIUM AND CHLORIDE VALUES OF PATIENTSWITH URINARY TRACT INFECTION

S.No.	Age	Sex	Name of the organism	Serum					Urine			
				Urea mg/100 ml	Creatinine mg/100 ml	Albumin g/100 ml	globulin g/100 ml	Total protein g/100 ml	Urea g/100 ml	Creatinine g/100ml	Protein mg/100 ml	pH
1.	16	Female	Pseudomonas aeruginosa	24	1.9	3.5	2.3	5.8	1.22	60	28	8.5
2.	25	Female	E.Coli	168	6.2	2.5	2.2	4.7	0.589	30	44	6.5
3.	83	Male	E.Coli	60	2.7	3.7	2.4	6.1	0.726	48	25	6.0
4.	63	Male	Pnoteus Vulyeus	82	2.2	3.5	2.4	5.9	0.803	45	15	8.8
5.	70	Male	E.Coli	53	1.9	4.0	2.2	6.2	0.921	73	44.	6.0
6.	22	Male	E.Coli	92	2.4	3.0	2.2	5.2	0.693	46	256	6.5
7.	21	Female	E.Coli	32	2.9	2.6	2.3	4.9	1.610	39	500	6.0
8.	55	Male	Klebsiela	88	2.4	3.4	2.2	5.6	0.780	48	250	7.0
9.	53	Female	E.Coli	58	2.3	3.5	2.1	5.6	1.120	37	64	6.0
10.	35	Male	E.Coli	130	4.0	3.2	3.0	6.2	0.750	40	40	6.5
11.	27	Male	E.coli	24	6.6	3.3	2.2	5.5	1.320	25	176	6.0
12.	67	Male	Pseudomonas aeruginosa	38	1.8	3.2	1.8	5.0	1.200	52	260	8.0
13.	60	Male	Pseudomonas aeruginosa	28	1.9	2.8	2.3	5.1	1.230	56	200	8.6
14.	65	Male	Klebsiela	209	6.0	3.0	2.3	5.3	0.503	28	40	8.5
15.	66	Male	E.Coli	63	2.2	2.5	2.1	4.6	0.763	42	600	8.6
16.	32	Male	Proteus vulgaris	28	2.0	4.4	2.5	6.9	1.190	94	20	8.5
17.	52	Male	Pseudomonas aeruginosa	170	5.0	3.8	2.3	6.1	0.831	35	45	9.0
18.	52	Male	E.coli	58	3.1	3.0	2.6	5.6	0.997	40	30	6.0

19	49	Male	E.Coli	158	4.5	3.4	.26	6.0	0.725	30	40	6.2
20	34	Female	Klehsiela	28	2.0	4.0	2.2	6.2	1.010	52	25	7.5
21.	74	Male	E.Coli	90	2.4	3.4	2.1	5.6	0.846	60	150	5.5
22.	20	Female	Klehsiela	42	2.9	3.8	2.4	6.2	1.310	38	20	7.0
23.	52	Male	E.Coli	58	2.0	4.2	2.1	6.3	0.910	92	15	6.0
24.	31	Female	Klehsiela	40	1.9	3.1	2.2	5.3	1.290	70	500	7.5
25.	23	Male	E.Coli	64	1.8	3.6	2.3	5.9	0.841	56	60	6.5
26.	85	Male	Klehsiela	92	2.7	2.9	2.4	5.3	0.750	47	45	6.5
27.	21	Male	Klehsiela	32	1.9	3.3	2.6	5.9	1.390	78	260	6.5
28.	19	Male	E.Coli	50	2.1	4.0	2.4	6.4	1.280	85	50	6.0
29.	58	Female	E.Coli	47	2.9	3.4	2.1	5.5	1.310	48	20	6.5
30.	17	Male	E.Coli	50	2.7	3.0	2.4	5.4	0.910	46	350	6.5
31.	25	Female	E.Coli	60	2.5	3.4	2.3	5.7	0.760	42	250	6.0
32.	32	Male	E.Coli	90	2.8	3.6	2.0	5.6	0.689	39	30	6.0
33.	24	Female	E.Coli	82	3.6	4.1	2.4	6.5	0.762	45	48	6.5
34.	69	Male	E.Coli	34	2.4	3.6	2.3	5.9	0.850	36	15	6.5
35	36	Male	Pnoteus Vulgaris	175	5.1	4.0	2.2	6.9	0.675	40	358	8.8
36.	32	Female	E.Coli	24	1.9	2.9	2.5	25.4	1.100	62	400	6.0

URINE

No.	Calcium mg/100ml	Phosphorus mg/100ml	Haemoglobin mg/100ml	Sodium mg/100 ml	Potassium mg/100ml	Chloride m
1.	10.0	54	-	92	32	100
2.	9.0	51	-	96	34	50
3.	8.0	46	-	86	32	54
4.	9.5	52	-	68	30	58
5.	9.5	58	2.1	60	32	46
6.	15.0	120	-	90	36	70
7.	10.0	53	12.4	120	40	92
8.	9.5	52	6.2	60	34	38
9.	13.0	68	1.1	120	42	108
10.	9.5	52	-	82	30	94
11.	14.0	70	3.9	90	32	82
12.	8.0	50	-	80	34	96
13.	13.0	59	-	72	30	50
14.	9.5	48	-	92	42	72
15.	8.0	50	3.2	116	42	125
16.	11.0	54	5.0	122	44	142
17.	14.0	120	-	84	34	66
18.	10.0	54	8.5	134	38	132
19.	9.5	52	4.3	90	34	110
20.	10.0	57	1.1	72	32	60
21.	13.5	69	-	80	30	78

No.	Calcium mg/100ml	Phosphorus	Haemoglobin	Sodium	Potassium	Chloride
22.	10.0	58	2.2	82	36	92
23	10.5	64	3.1	126	40	136
24.	12.0	65	-	88	36	64
25.	13.0	80	-	92	36	84
26.	9.0	52	-	74	32	78
27.	11.0	60	1.8	90	38	78
28.	13.0	75	-	92	40	88
29.	9.0	58	-	80	32	86
30.	9.5	58	-	60	32	48
31.	13.5	70	-	68	30	58
32.	8.5	49	-	124	42	132
33.	9.5	55	5.7	84	34	92
34.	11.5	65	-	110	42	142
35.	10.5	55	-	80	32	82
36.	9.5	52	1.8	106	36	120.

APPENDIX XI

SERUM AND URINARY UREA, CREATININE PROTEIN AND URINARY pH? CALCIUM, PHOSPHORUS, SODIUM, POTASSIUM AND CHLORIDE VALUES IN CONTROLS

S.No.	Age	Sex	SERUM					URINE							
			Urea mg/100 ml	Creatinine mg/100ml	Albumin mg/100ml	Globulin g/100ml	Total g/100 ml	Urea g/100 ml	Creatinine mg/100 ml	Protein mg/100ml	pH	Calcium mg/100 ml	Phosphorus mg/100ml	Sodium m.eqll	Potasium m.eqll	Chloride m.eql
1.	25	Female	30	1.9	4.9	2.9	7.8	1.12	64	8	6	9.5	52	126	46	153
2.	83	Male	34	1.8	5.2	2.4	7.6	1.08	68	15	5.5	7.5	48	116	40	120
3.	63	Male	38	1.8	5.4	2.6	8.0	1.29	75	20	5.8	9.0	50	109	42	110
4.	70	Male	32	2.0	4.8	2.4	7.2	1.36	78	15	6.2	9.5	50	100	36	100
5.	22	Male	42	1.7	5.8	2.6	8.4	1.45	58	12	6.0	11.0	58	128	42	130
6.	21	Female	30	1.9	5.3	2.8	8.1	1.31	75	20	6.0	9.5	54	110	42	136
7.	55	Male	36	2.0	5.3	2.5	7.8	1.25	80	18	6.2	10.0	50	124	44	160
8.	53	Female	34	1.7	6	3	9.0	1.28	56	15	6.4	8.0	48	112	38	126
9.	27	Male	24	2.0	5.3	2.8	8.1	1.29	84	14	6.0	10.5	56	122	42	136
10.	67	Male	38	2.1	5.2	2.9	8.1	1.23	87	20	6.0	8.0	49	126	40	148
11.	60	male	36	1.8	4.5	2.5	7.0	1.30	68	20	5.8	9.5	51	134	38	142
12.	65	Male	40	2.1	5.0	2.5	7.5	1.43	89	15	6.0	9	48	109	42	110

13.	66	Male	40	2.1	5.2	2.8	8.0	1.46	86	16	6.0	8.5	48	112	40	130
14.	32	Female	37	1.9	5.2	2.8	8.0	1.45	75	12	5.8	10.5	54	122	42	136
15.	52	Male	26	1.8	5.0	2.5	7.5	1.27	84	14	5.8	10.5	52	118	42	150
16.	52	Male	28	1.9	5.6	2.6	7.6	1.27	78	15	6.2	10.0	55	120	40	132
17.	49	Female	32	1.6	5.3	2.6	7.9	1.37	58	13	6.0	11.0	57	100	38	118
18.	74	Male	34	1.9	5.0	2.5	7.5	1.35	82	18	6.0	8.5	47	146	48	168
19.	20	Female	28	1.7	5.3	2.8	8.1	1.25	56	10	6.5	10.5	58	110	42	136
20.	54	Male	34	1.8	5.1	2.7	7.8	1.38	74	20	5.8	11.0	62	120	40	132
21.	31	Female	32	1.8	5.6	2.8	8.0	1.32	64	15	5.6	10.0	53	134	38	148
22.	23	Male	28	1.7	5.6	2.7	8.3	1.36	58	15	6.0	11.0	59	128	42	130
23.	85	Male	34	1.9	4.9	2.5	7.4	1.28	66	17	6.2	8.0	48	116	42	130
24.	21	Male	32	2.0	5.3	2.8	8.1	1.29	80	12	6.5	11.5	62	116	42	128
25.	19	Male	28	2.0	5.8	2.9	8.6	1.20	84	16	6.0	11.0	63	128	42	130
26.	58	Female	26	1.8	5.7	3.2	8.9	1.38	58	20	6.0	8.5	55	116	48	140
27.	25	Female	30	1.7	5.2	2.6	8.8	1.12	60	14	6.2	10.0	52	120	38	136
28.	32	Male	37	1.8	5.6	2.8	8.4	1.45	72	18	5.8	8.0	48	126	46	153
29.	24	Female	30	1.8	5.6	2.9	8.5	1.15	68	10	6.0	9.5	56	122	46	136
30.	39	Male	42	1.6	5.6	2.6	8.0	1.48	60	15	5.6	9.0	53	126	46	153
31.	16	Female	36	1.9	5.6	2.8	8.4	1.2	58	20	6.2	9.5	50	120	40	132