



Effects of *Escherichia coli* Infection on Some Serum and Urine Biochemical Parameters in Premenopausal and Postmenopausal Patients

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Abstract

The changes in some serum and urine biochemical parameters in *Escherichia coli* infected premenopausal and postmenopausal patients were studied. Blood specimens as well as early morning midstream urine specimens were collected from forty premenopausal patients aged 13-15 years, forty postmenopausal patients aged 45-60 years and twenty control subjects with no evidence of *Escherichia coli* infection aged 20-40 years. These specimens were collected into plain (non anticoagulated) and sterile universal bottles respectively. The following blood biochemical parameters: creatinine, total protein, albumin, globulin and C-reactive protein were quantitatively measured. The results showed significant differences of C-reactive protein in both the premenopausal and postmenopausal patients as compared with the control subjects while the other blood biochemical parameters: creatinine, total protein, albumin and globulin showed no significant differences. The urine dipstick results showed positive protein reaction in 30% of the premenopausal patients, 70% of the postmenopausal patients and 0% of the control subjects while positive nitrite reaction of 20%, 80% and 0% were obtained in the premenopausal patients, postmenopausal patients and control subjects respectively. The pH cut off mark of ≥ 8.0 showed 50%, 85% and 0% in the premenopausal patients, postmenopausal patients and control subjects respectively. However, the other urine biochemical parameters: blood, urobilinogen, bilirubin, ascorbic acid, ketones and glucose showed negative reactions in the premenopausal, postmenopausal and control subjects respectively. In conclusion, this study has shown that serum C-reactive protein concentration is significantly elevated in both the premenopausal and postmenopausal patients infected with *Escherichia coli* while dipstick urine test results for protein and nitrite are significantly altered in the postmenopausal patients only.

Keywords: *Escherichia coli* infection, serum biochemical parameters, urine biochemical parameters, premenopausal, postmenopausal.

Introduction

Escherichia coli is a genus of gram negative, motile, rod like bacteria that has the ability to ferment carbohydrate, usually with the production of gas and is found in the intestines of human beings and many animals.

Escherichia coli, a lactose fermenting specie is usually not harmful, but some strains cause gastrointestinal infections. Its colonies which are about 1.25mm in diameter are usually yellow with slight deeper coloured centre¹. This microorganism causes about 80-85% of the infections associated with urinary tract² with women more at risk than men because they have shorter urethra which is closer to the anus³.

The first documented description of urinary tract infections was in the "Ebers Papyrus" which dated as far back C.1550BC⁴ and described by the Egyptians as "sending forth heat from the bladder"⁵. There was no reliable treatment for this infection until antibiotics were developed and readily available in the 1930s. However, before this time the recommended treatment was herbs, bloodletting and rest⁴. Various methods have been used

to screen for bacteriuria or to identify invasive urinary tract infection, these include microscopic examination of voided urine (centrifuged or uncentrifuged), biochemical testing of urine and culture technique⁶.

Indeed urinary tract infections occur when microorganisms adhere, multiply and persist in a portion of the kidneys, ureters, bladder or urethra. Infection of the bladder is known as cystitis while infection of the kidney is known as pyelonephritis. The symptoms of the bladder infection include painful urination, frequent urination or both² which vary from mild to severe⁷, these symptoms however, last for an average of six days in healthy women⁸ while flank pain, fever or nausea are the symptoms associated with pyelonephritis⁷. It should be noted that these symptoms are not often associated with the elderly⁹. However, their presentation shows some degree of changes in mental status, vague with incontinence or fatigue⁷. The urine of urinary tract infected subjects may rarely appear bloody or contain visible pus cells¹⁰. For several years interest has been shown in studies on urinary tract infections probably because of its health and social implications. However, studies showing the effects of *Escherichia coli* infection on blood and urine

biochemical parameters in premenopausal and postmenopausal patients are rarely available hence this study which was aimed at determining the changes in some blood and urine biochemical parameters in premenopausal and postmenopausal patients infected with *Escherichia coli*.

Methodology

Forty premenopausal patients aged 13-15 years as well as forty postmenopausal patients aged 45-60 years who were attending outpatients clinics in some hospitals in Yenagoa, Bayelsa State of Nigeria whose early morning midstream urine specimens upon culture grew $\geq 10^5$ bacteria/ml pure growth of *Escherichia coli* were recruited for this study.

All these patients had symptoms that led to attendance of outpatients clinics in some hospitals in Yenagoa Bayelsa State of Nigeria with the subsequent request for urine m/c/s, However, in this study, no attempt was made to analyze their symptoms, but the confirmation of their menopausal status as either premenopausal or postmenopausal as well as their ages were ascertained by oral interview.

The apparently healthy subjects (control) consisted of twenty; aged 20 to 40 years and who in the course of this study were found not to have urinary tract infections clinically as well as in the laboratory investigations performed and were also not on any therapy associated with antibiotics.

After seeking the consents and approval of these patients and control subjects, 5ml blood specimens as well as early morning midstream urine specimens were collected from each of them into plain (non anticoagulated) bottles and clean, sterile universal bottles respectively. The blood specimens were allowed to clot, carefully retracted and spun with the obtained serum used for the quantitative measurement of the following biochemical parameters using the specified methods: creatinine, Jaffe reaction method previously described by Jaffe in 1886 and modified by Randox Laboratory kit manual, Ardmere, United Kingdom¹¹, total protein, Biuret method as described in Randox Laboratory kit manual, Ardmere, United Kingdom¹², albumin, Bromocresol green method as described in Randox Laboratory kit manual, Ardmere, United Kingdom^{13,14}, globulin Sandle and Alderson method as described by Cynthia C.C.et.al.¹⁵ and C-reactive protein, Latex turbidometry method as described by Spinreact Diagnostic kit manual, Spain¹⁶ while the urine specimens were cultured on cystine lactose electrolytes deficient (CLED) agar using a standard procedure and incubated aerobically at 37°C with the results of the cultured urine specimens read at 24 hours of incubation and *Escherichia coli* of $\geq 10^5$ colony forming units/ml established. Patients with contaminated urine having the presence of at least two different microorganisms in the urine specimens were excluded. The antimicrobial sensitivity test was performed and antimicrobial therapy recommended for these patients according to the antimicrobial sensitivity results.

Routine urinalysis (urine biochemical parameters) were estimated shortly after the specimens were cultured using the in vitro diagnostikum (dipstick) method as described in meditest combi 9 laboratory kit manual, Duren.

Statistical Analysis: The results were expressed as the mean and standard deviation while the differences between the subjects were assessed using the student's "t" tests. The results were considered statistically significant at $p < 0.05$

Results and Discussion

In this study, the mean values of the estimated blood biochemical parameters as well as the qualitative reactions of the urine biochemical parameters in both the premenopausal and postmenopausal patients infected with *Escherichia coli* were compared with that of the apparently healthy (control) subjects.

A summary of the results of blood biochemical parameters in the control subjects as compared with that of the premenopausal and postmenopausal patients infected with *Escherichia coli* is as shown in table-1. The results showed significant differences of C-reactive protein in both the premenopausal and postmenopausal patients as compared with that of the control subjects while other blood biochemical parameters: creatinine, total protein, albumin and globulin showed no significant differences in both the premenopausal and postmenopausal patients as compared with that of the control subjects. Table-2 shows the number and percentage of apparently healthy (control) subjects with positive urine biochemical reaction compared with the infected premenopausal and postmenopausal patients. The results showed that 30% of the premenopausal patients infected with *Escherichia coli* had positive protein reaction while 70% of the postmenopausal patients infected with *Escherichia coli* had positive protein reaction. However, none of the apparently healthy (control) subjects were found to have positive protein reaction.

This result went further to show positive nitrite reaction in 20% of the premenopausal patients infected with *Escherichia coli* and 80% of the postmenopausal patients infected with *Escherichia coli*. However, none of the apparently healthy (control) subjects had positive nitrite reaction. In addition, 50% of the premenopausal patients infected with *Escherichia coli* were found to have pH value ≥ 8.0 while the postmenopausal patients infected with *Escherichia coli* had 85%. However, none of the apparently healthy (control) subjects had pH value ≥ 8.0 . All the other urine biochemical parameters: blood, urobilinogen, bilirubin, ascorbic acid, ketones and glucose showed negative reactions in the *Escherichia coli* infected premenopausal and postmenopausal patients as well as in the apparently healthy (control) subjects.

Discussion: The results from this study showed that the mean values of serum creatinine in the premenopausal and postmenopausal patients infected with *Escherichia coli* were not statistically significant ($p > 0.05$) as compared with that of the

control subjects as shown in table-1. This finding shows that *Escherichia coli* infection isolated from urinary tract is not in any way associated with elevation in serum creatinine concentration.

This work further revealed that there was no statistical significant differences ($p > 0.05$) in the mean values of serum total protein in the premenopausal and postmenopausal patients infected with *Escherichia coli* as compared with that of the control subjects as shown in table-1. This finding strongly suggests that patients with *Escherichia coli* infection isolated from urinary tract are not likely to have increase in serum concentration of total protein.

The mean value of serum albumin in the *Escherichia coli* infected premenopausal and postmenopausal patients as compared with that of the control subjects showed no statistical significant difference ($p > 0.05$) as shown in table-1. It is suggested from this finding that *Escherichia coli* infection isolated from urinary tract is not associated with an elevation in serum albumin concentration.

The mean values of serum globulins in the premenopausal and postmenopausal patients infected with *Escherichia coli* as compared with that of the control subjects showed no statistical significant difference ($p > 0.05$) as shown in table-1. This finding is suggestive that *Escherichia coli* infection isolated from

urinary tract is not associated with an elevation in serum globulins concentration.

The results of this work showed that the mean values of serum C-reactive proteins in the premenopausal and postmenopausal patients infected with *Escherichia coli* were statistically significant ($p < 0.05$) as compared with that of the control subjects as shown in table-1. These findings as confirmed in this study may be caused by inflammation within the urinary tract of these patients as a result of the invasive infection of *Escherichia coli*.

The results of this study went further to show positive nitrite reaction in the urine specimens of 20% and 80% of the premenopausal and postmenopausal patients infected with *Escherichia coli* respectively while none of the apparently healthy (control) subjects showed positive reaction.

This results showed a considerable significant difference in the sensitivity of urinary nitrite as an indicator of urinary tract infection most particularly *Escherichia coli*. Considering the low percentage of positive urinary nitrite result in the premenopausal patients and high percentage in the postmenopausal patients, the significance of this test is therefore limited to postmenopausal patients infected with *Escherichia coli* as against the premenopausal patients in which it is fairly insignificant.

Table-1
Results of the serum biochemical parameters for the control subjects compared with the premenopausal and postmenopausal patients infected with *Escherichia coli*

Parameters measured	Control(n=20)	Premenopausal patients infected with <i>E.coli</i> (n=40)	Postmenopausal patients infected with <i>E.coli</i> (n=40)	Remark
Creatinine(mg/dl)	0.77 ± 0.13	0.75±0.17	0.73±0.15	NS
Total protein(g/l)	71.20±4.75	68.35±5.51	68.42±5.60	NS
Albumin(g/l)	40.08±1.41	39.20±1.60	39.27±1.68	NS
Globulin(g/l)	31.12±4.25	29.15±5.00	29.15±5.02	NS
Crp(mg/l)	0.23±0.05	10.97±3.10	12.53±3.47	S

Values are means and S.D. of triplicate determinations at $p < 0.05$, NS represents not significant, S represents, significant, n represents the number of subjects, Crp represents C-reactive protein

Table-2
Number and percentage of apparently healthy (control) subjects with positive urine biochemical reactions compared with the infected premenopausal and postmenopausal patients

Parameters	Control (n=20)	Premen (n=40)	Postmen (n=40)
Blood	00(00)	00(00)	00(00)
Urobilinogen	00(00)	00(00)	00(00)
Bilirubin	00(00)	00(00)	00(00)
Protein	00(00)	30 (12)	70(28)
Nitrite	00(00)	20 (8)	80(32)
Asc.acid	00(00)	00(00)	00(00)
Ketones	00(00)	00(00)	00(00)
Glucose	00(00)	00(00)	00(00)
p ^H	00(00)	50 (20)	85(34)

Values are in percentages, no of subjects with positive reactions are in parenthesis, p^H cut off mark are values ≥ 8.0 , n is number of subjects, premen is premenopausal, postmen is postmenopausal

This finding is in agreement with some researchers^{7,9,10} who in their various researches reported that false positive urinary nitrite results are rare and that the sensitivity of the test is reported to be as low as 21-59% by some authors^{18,19} and as high as 80-93% by Scheifele D.W. et. al.²⁰ with those that reported low advising against the use of the test in screening patients at risk of urinary tract infections, while those that reported high advocating for the use of the test in screening patients at risk of urinary tract infections.

A systematic view studies by Carter I.J. et.al.¹⁷ reported an association between symptomatic urinary tract infections and proteinuria. However, in this study the dipstick urine tests showed protein positive results in 30% premenopausal patients infected with *Escherichia coli* as against 70% postmenopausal patients infected with *Escherichia coli*, a situation which suggests a great link between *Escherichia coli* infection and urinary protein in postmenopausal patients as against the premenopausal patients. This finding agrees with the report of Simerville J.A. et.al.²¹ who in their work reported urinary tract infections to be commonly associated with proteinuria within the range of 63-83% in culture confirmed cases of urinary tract infections.

This study went further to establish an insignificant link between *Escherichia coli* infection and urinary protein in premenopausal patients. It is presumed from this study that proteinuria in premenopausal patients infected with *Escherichia coli* is not a reliable screening test for bacteriuria.

It was shown in this study that 50% of the premenopausal patients infected with *Escherichia coli* had a pH value ≥ 8.0 as against 85% of the postmenopausal patients infected with *Escherichia coli* while none of the apparently healthy (control) subjects had a pH value ≥ 8.0 . It is presumed from this study that the high percentage (85%) of pH value ≥ 8.0 in the postmenopausal patients may be as a result of the breakdown of urea by *Escherichia coli*. Thus, from this study the higher percentage of urinary protein, nitrite and pH value ≥ 8.0 as found in the postmenopausal patients as compared with that of premenopausal patients is presumed to be due to the decreased level of estrogen which is associated with menopause a situation which puts them at risks of contacting urinary tract infections as a result of the loss of their protective vaginal flora as reported by Dielubanza E.J. et.al.³ in their research work.

Conclusion

In conclusion, this present study has proved that *Escherichia coli* isolated from urinary tract infection influences significant elevation of serum C-reactive protein concentrations in both premenopausal and postmenopausal infected subjects while significant positive dipstick urine test results for nitrite and protein are influenced in postmenopausal infected subjects only.

Recommendations: The following recommendations are suggested: Preliminary biochemical test, most importantly serum C-reactive protein (Crp) estimation be carried out in every confirmed case of *Escherichia coli* urinary tract infection and the concentration be brought to normal level upon the finding of a significant elevation in the patients under investigation.

Medical practitioners should not rely completely on urinary nitrite and protein results in premenopausal patients showing some symptoms of urinary tract infections while making conclusion for bacteriuria, thus it is pertinent to request for another midstream urine specimen for m/c/s in order to properly establish and identify bacteriuria as the potential cause of the positive nitrite and protein.

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