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Variation in Progesterone Levels and Urinary Tract Infections in Pregnant Women Attending Moi Teaching and Referral Hospital, Eldoret, Kenya

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Abstract

Background and Aims: Urinary tract infections (UTIs) are common among pregnant women and major predisposing factors for pyelonephritis linked to obstetrical complications including preterm labour and low infants' birth weights. This study sought to determine the relationship(s) between pregnancy trimesters, UTIs and changes in progesterone levels among pregnant women. **Materials and Methods:** The study was conducted in 2016 at Moi Teaching and Referral Hospital (MTRH) antenatal clinic which is a referral facility that attends to patients from most Counties in western region of Kenya. A cross-sectional study design was used to collect blood and urine specimens from 78 participants. Blood was used to determine progesterone levels using ELISA technique and urine cultures with bacterial colony counts $\geq 10^5$ were appropriately identified to species level. Trimester periods and participants' demographic information were obtained using a structured questionnaire. **Results:** Culture results showed that the most abundant bacterial species isolated in urine from the pregnant women was *Escherechia coli* (63.7%). The more affected age-group was women between 30 - 39 years during trimester three, suggesting that bacterial colonization of genital track occurred more frequently in older compared to the younger women. There was an exponential increase in progesterone levels among the pregnant women during trimester three compared to other trimesters, although these increases occurred independent of age. However, high levels of progesterone among pregnant women in third trimester corresponded with increased number of *E. coli* causing UTI. **Conclusion:** The results showed that proge-

sterone levels increase with trimester and the most prevalent bacteria associated with this was *E. coli* even though age and increase in progesterone levels had no significant impact on *E. coli* infection.

Keywords

Urinary Tract Infection, Progesterone, Trimester, *Escherichia coli*

1. Introduction

Urinary tract infection (UTI) defined as the presence of uropathogens greater than 10^5 CFU/ml in urine culture is a common health problem among pregnant women [1]. UTIs can present symptomatically [2] or asymptomatically [3], usually affecting 18% and 13% pregnant women respectively [3]. Asymptomatic bacteriuria is a major predisposing factor for pyelonephritis, a condition linked to obstetrical complications, preterm labor and low infants' birth weight [3].

UTIs are common causes of maternal and fetal morbidity and mortality [4]. Bacteriuria is predisposed to anatomical and physiological changes imposed on the urinary tract by the hormone progesterone [5]. Progesterone is necessary for implantation of the fertilized egg in the uterus and for maintaining pregnancy [6]. The adjustments driven by progesterone hormones increase in concentration during pregnancy when the maternal immune system is lowered to enable immune tolerance towards paternal antigens expressed on fetal cells [7]. Lowered immunity promotes the growth of pathogens including *E. coli* in the urinal system of pregnant women.

Increased risk of infection among pregnant women has been attributed to physiological changes, but little emphasis has been made to determine the association between *E. coli* occurrence and the progesterone hormone levels [5].

The main aim of the current study was to determine the relationship between pregnancy trimesters, changes in progesterone levels and occurrence of UTIs detected in the urine among pregnant women attending antenatal clinic at Moi Teaching and Referral Hospital.

2. Materials and Methods

2.1. Study Area and Study Population

The study was conducted among pregnant women attending antenatal clinic at Moi Teaching and Referral Hospital (MTRH) in Eldoret, which is situated in the mid-west of Kenya's Rift Valley and shares common borders with Trans Nzoia County to the North, Elgeyo Marakwet County to the East, Baringo County to the South East, Kericho County to the South, Nandi County to the South West and Kakamega County to the North West. Moi teaching and referral hospital is a referral hospital facility with a catchment population from western Kenya. The facility was purposely chosen because of its elaborate clinic for mother to child

infection prevention programme, good patient flow and increased urinary tract infections in pregnant women.

2.2. Study Design

A cross sectional design was used and sample size was calculated using the formula of Agresti [8] and Agresti [9] which determined a sample size of 78 participants. All women attending antenatal clinic at MTRH were randomly selected and those who consented and had not used antibiotics during the previous one week were recruited for the study. Every 8th consenting pregnant woman who met inclusion criteria in age groups 15 - 19 (3), 20 - 29 (46), 30 - 39 (25), and 40 - 49 (4) was recruited for the study until the sample size was achieved thus 26 participants for each trimester).

2.3. Sampling Frame

The patients flow in MTRH antenatal clinic for 20 working days is approximately two hundred. The sampling frame of three months was used and 78 samples of both urine and blood were collected by appropriate probability sampling method.

2.4. Sampling Technique

Probability sampling method was used and 78 blood and urine samples were collected at Intervals for 60 working days. Approximately 600 samples are collected at antenatal clinic in 60 working days and 78 samples were required for the study, therefore a sample interval of 8 was used. Every 8th pregnant woman who met the inclusion criteria and consented to participate was sampled until 78 participant were sampled

2.5. Sample Size Determination and Sampling

The sample size required for a logistic regression, in which the predictor is quantitative is given by (Agresti, 2002, 2007).

$$n = \left(\frac{\left[z_{\alpha} + z_{\beta} \exp\left(-\lambda^2/4\right) \right]^2 \left(1 + 2\bar{\pi}\delta\right)}{\bar{\pi}\lambda^2} \right)$$

where

$$\delta = \left(\frac{1 + \left(1 + \lambda^2\right) \exp\left(5\lambda^2/4\right)}{1 + \exp\left(\lambda^2/4\right)} \right) = \left(\frac{1 + (1 + 1.06) \exp(5 \times 1.06/4)}{1 + \exp(-1.06/4)} \right) = 5.0$$

Thus

$$n = \left(\frac{\left[1.645 + 0.84 \times \exp\left(-1.06/4\right) \right]^2 \left(1 + 2 \times 0.4 \times 5.0\right)}{0.4 \times 1.06} \right) = 62$$

where λ is Lambda, α Alpha, β Beta δ delta and n population size

This sample size was sufficient to model the relationship between the quantitative predictor x, here progesterone levels, and the outcome, presence of *E. coli*. The incidence of *E. coli* from a study conducted at the University of Nairobi was 40% [10]. For simplicity and reasons for computing the sample size, it was assumed that this prevalence value was at an average progesterone level. Higher progesterone levels have been associated with higher incidence of *E. coli* [10]. Using the incidence of 40% from the study conducted in Nairobi, a sample size that allowed the test to be sensitive to at least a 25% difference in the prevalence of *E. coli* was required, that is, to increase to 65% from the population prevalence, at one standard deviation increase in the predictor, progesterone levels. The odds of *E. coli* occurrence at the mean value of progesterone is 0.40/0.60 = 0.67, and the odds of *E coli* occurrence at a unit standard deviation above the mean are 0.65/0.35 = 1.9. This means that there is odds ratio hence, the chance of type II error in a test. Thus, the sample size required for one predictor is 62. However, for a multivariate logistic regression model where we have other covariates to adjust for in the model, an assumption was made about the correlation between progesterone and the rest of the covariates. It was assumed that R^2 is 0.2 for moderate correlation. To get the sample size that was sufficient to study the relationship between the outcome and the anticipated factors division of the sample size was obtained above by $(1 - R^2)$, which gave 78 as the number of participants

2.6. Inclusion and Exclusion Criteria

All pregnant women attending antenatal clinic at MTRH who consented to the study were recruited after fulfilling the inclusion criteria. For instance, consenting pregnant women who had not used antibiotics during the previous one week were recruited. However, pregnant women who had used antibiotics during the same period together with those who did not consent to the study were excluded.

2.7. Urine Specimen Collection, Processing and Analysis

Study participants were advised to collect mid stream urine and were provided with a sterile screw capped urine container. About 0.001 ml urine specimens were transferred using calibrated loop onto cysteine lactose electrolyte deficient (CLED) agar media [11] and incubated aerobically at 37˚C for 24 hours. Any microorganism isolated with colony counts of 105 CFU/ml of urine was considered positive for urinary tract infection [11]. Bacteria were identified to species level using Gram staining and biochemical tests.

2.8. Blood Specimen Collection and Processing

Blood was collected from each participant by a phlebotomist using standard procedure. Plasma was obtained from 2 ml of blood centrifuged at 3000 - 5000 rpm at room temperature and aliquoted into cryovials and used to determine

progesterone levels by ELISA technique [12]. The samples were analysed in duplicates and the mean levels were determined and recorded.

2.9. Progesterone Levels Determination

Blood specimens in microtiter plates containing progesterone hormone conjugate were shaken and incubated at room temperature for an hour. Addition of substrate generated hormone-substrate complex with an optimal color after 30 minutes. The extent of color development was inversely proportional to the amount of progesterone in the specimen and was determined by measuring the absorbance (at 450 nm) of the specimens against the standards using a microtiter plate reader (*BIOTEK S/N* 200136) [12].

2.10. Data Management and Analyses

Data analysis was done using STATA version 13 SE. Categorical variables were summarized as frequencies with corresponding percentages. Continuous variables were summarized by mean and standard deviation (SD). Differences between two normally distributed continuous variables (such as mean progesterone levels during different trimesters) were evaluated using two-sample t-test while those with discontinuous variables (such as *E. coli* occurrence or non occurrence) were tested by chi-square technique.

3. Results

Out of the 78 urine specimens that were tested, 11 were found to be positives for bacterial pathogens. The most frequent bacterial species that was observed was *Escherechia coli* (63.7%) and the least was *Klebsiella* spp (9.1%) of positive samples as shown in Table 1. However, the overall sampled population prevalence of *E. coli* was 9.3% (Table 3).

The mean progesterone levels at first and second trimesters were within normal ranges; 9 - 47 ng/ML and 17 - 146 ng/ML for first and second trimester respectively (Table 2). However, there was exponential increase of progesterone hormone from trimester one (1) to trimester three (3), with significant differences ($p < 0.5$) in mean plasma progesterone concentration among pregnant women during the different trimesters. The mean progesterone levels were

Table 1. Bacterial pathogens identified in the urine of pregnant women attending antenatal clinics at MTRH.

Type of bacterial isolate	No. Positive	Percent of positive (%)
Escherechia coli	7	63.7
Enterococcus fecalis	2	18.2
Pseudomonas aeruginosa	1	9.1
Klebsiella spp	1	9.1
Total	11	100

Table 2. Progesterone concentrations (ng/ML) by trimester in pregnant women attending antenatal clinic at MTRH. Means followed by different letters within a column are significantly different at $p < 0.05$.

	Trimester One	Trimester Two	Trimester Three
	44.071 ± 2.31^{c}	58.167 ± 10.12^{d}	104.712 ± 20.13^{b,c}
	8.411 ± 1.18^{a}	52.131 ± 9.64^{d}	60.408 ± 12.57^{b}
	9.494 ± 1.24^{a}	12.61 ± 2.87^{b}	59.494 ± 11.41^{b}
	47.552 ± 5.54^{c}	5.895 ± 1.27^{a}	71.383 ± 11.39^{b}
	9.503 ± 1.27^{a}	39.724 ± 7.98^{c}	329.373 ± 31.97^{e}
	10.972 ± 3.11^{a}	35.135 ± 9.12^{c}	77.995 ± 11.78^{b}
	15.987 ± 4.23^{a,b}	10.056 ± 2.11^{b}	590.844 ± 27.81^{g}
	12.383 ± 3.76^{a}	43.625 ± 10.75^{c}	56.937 ± 5.31^{b}
	9.485 ± 2.44^{a}	80.253 ± 12.97^{e}	157.910 ± 18.95^{c}
	9.640 ± 2.99^{a}	75.859 ± 13.11^{e}	432.309 ± 24.71^{f}
	8.955 ± 2.54^{a}	31.634 ± 3.28^{c}	92.013 ± 7.13^{b}
	9.185 ± 3.13^{a}	43.670 ± 8.76^{c}	66.238 ± 8.34^{b}
	5.463 ± 1.03^{a}	49.562 ± 9.19^{c}	80.671 ± 9.47^{b}
	8.814 ± 3.18^{a}	19.975 ± 5.14^{b}	279.059 ± 45.67^{d}
	9.767 ± 2.12^{a}	105.391 ± 20.76^{e}	393.992 ± 41.34^{e}
	10.971 ± 3.07^{a}	25.996 ± 3.19^{c}	946.278 ± 57.32^{g}
	11.498 ± 4.20^{a}	59.027 ± 18.21^{d}	83.759 ± 21.37^{b}
	12.052 ± 3.56^{a,b}	37.852 ± 10.78^{c}	55.759 ± 10.91^{b}
	11.535 ± 4.06^{a}	39.335 ± 9.13^{c}	301.774 ± 45.31
	20.713 ± 5.58^{b}	103.564 ± 28.45^{e}	57.657 ± 5.78^{b}
	9.996 ± 2.96^{a}	37.485 ± 7.11^{c}	8.672 ± 2.43^{a}
	11.963 ± 3.75^{a}	11.235 ± 2.91^{b}	143.322 ± 29.34^{c}
	20.218 ± 4.48^{b}	14.084 ± 3.72^{b}	237.497 ± 49.12^{d}
	10.369 ± 2.94^{a}	15.522 ± 2.88^{b}	704.402 ± 63.43^{h}
	9.089 ± 1.77^{a}	36.485 ± 7.35^{c}	90.834 ± 17.45^{b}
	9.885 ± 2.23^{a}	77.318 ± 5.57^{e}	34.215 ± 10.23^{b}
F-Value	**9.771**	**2.348**	**473**
P-Value	**0.000****	**0.000****	**0.000****

(Normal ranges: 9 - 47 ng/ML, 17 - 146 ng/ML, 43 - 300 ng/ML).

above normal range (43 - 300 ng/ML) during the third trimester as shown in Table 2.

The distribution of *E. coli* during the three gestation periods (trimesters) is shown in Table 3. There was a higher percentage of *E. coli* infection during the

third trimester (12%) compared to second and first trimesters, which had 8% infections each. However, chi-square statistics showed that the observed differences were not significant ($p > 0.05$).

The prevalence of *E. coli* infection in relation to age is shown in Table 4. A majority (58.9%) of participants in this study were pregnant women between age-group 20 - 29 years. However, pregnant women of age group 30 - 39 years had the highest incidence of *E. coli* infection (16 %) followed by those in age group 20 - 29 years who had 6.5% *E. coli* infection. There was no *E. coli* incidence in pregnant women of age groups 15 - 19 years and 40 - 49 years probably due to the fewer numbers of these age-groups among the participants. However, Pearson chi-square test showed insignificant association ($p > 0.05$) between the age-groups and *E. coli* occurrence (Table 4).

4. Discussion

Majority of pregnant women who participated in this study were within the child bearing age of between 20 - 39 years which is consistent with the report of Campbell [13] who conducted interviews among senior women, majority of whom reported that pregnancy in early age (≤19 years) is shunned by the society as it undermines the future of young girls. Similarly, fewer pregnant women of 40 years and above were encountered since at this advanced age, women experience infertility and other social and health complications, which deter them from getting pregnant [14].

The most prevalent bacteria isolated in urine of the pregnant women attending ANC at MTRH was *Escherechia coli*. *Pseudomonas aeruginosa* and *Kleibsiella spp.* were infrequently encountered. This could be because uropathogenic *E. coli* expresses a multitude of virulence factors to break the inertia of the mucosal

Table 3. Distribution of *E. coli* frequency during the three gestation periods (trimesters).

Trimester	No. Specimens	Positive for *E. coli* (%)	χ^2	*P*-value
First	26	2(8%)		
Second	26	2(8%)		
Third	26	3(12%)	49.41	0.859
Total	**78**	**7(9.3%)**		

Table 4. Prevalence of *E. coli* in pregnant women in relation to age.

Age group	No. of specimens	*E. coli* positive (%)	χ^2	P-value
15 - 19	3 (4)	0		
20 - 19	46 (58.9)	3 (6.5)	2.734	0.431
30 - 39	25 (32.1)	4 (16)		
40 - 49	4 (5)	0		
Total	**78**	**7**		

barrier, and can persist within the urinary tract serving as a reservoir for recurrent infections and serious complications. These results are consistent with those of Zen *et al.* [15] and Ronald [5] who found that *E. coli* represents 80.0% of bacterial isolates in bacteriuria while the current study *E. coli* result was at 63.7% of bacterial isolates in bacteriuria. However, Tadesse *et al.* [16] observed a lower prevalence rate (10.4%) of *E. coli* which is comparable to our result of 9.3% *E. coli* prevalence among all pregnant women in the study. *Pseudomonas spp.* was isolated in only one case in the present study. The very low growth of *Pseudomonas spp.* could be attributed to the fact that all the investigated participants were from the outpatient department and *Pseudomonas spp* is more commonly acquired as a nosocomial infection following prolonged hospital stay. Hamdan *et al.* [17] and Ronald [5] also reported similar low prevalence results for this species.

There was an exponential increase in progesterone hormone among study participants from trimesters one to trimester three, which is consistent with the observation that progesterone levels increase as pregnancy progresses [3]. The increase was progressive from the first trimester, reaching a peak during the third trimester of pregnancy as demonstrated by our data (**Table 2**). Progesterone is primarily produced by the placenta during pregnancy [4]. Progesterone levels during pre-ovulatory phase of the menstrual cycle are relatively low, and it rises after ovulation and is elevated during the luteal phase [10]. If pregnancy occurs, human chorionic gonadotropin is released maintaining the corpus luteum, allowing it to maintain appropriate levels of progesterone [4].

The results of the current study show that the frequency of *E. coli* was higher during the third trimester compared to the first and second trimesters. This probably occurred because of weakening of immune systems of the body as pregnancy progresses. Jennifer *et al.* [18] reported a similar occurrence and observed that changes due to pregnancy results in increase in bladder volume while detrusor tone decreases, and leads to relaxation of ureteric smooth muscles causing ureter dilatation, which could lead to urinary stasis hence supporting the colonization of the bladder by bacterial organisms. Onoh *et al.* [19] and Emiru *et al.* [20] also found an increased frequency of *E. coli* during the third trimester compared to the first and second trimesters. This may also be as a result of the pressure effect of a bigger uterus on the ureter at the third trimester, and also the increasing smooth muscle relaxing effect of pregnancy hormones and the pressure on the bladder which may lead to stasis of urine which encourage bacteria multiplication. Onoh *et al.* [19] also reported a higher prevalence of *E. coli* infection in the second trimester compared to the third trimester. However, this observation needs further research to confirm. Furthermore, several individual and socio-economic factors can impact on exposure and susceptibility to infection. The possible confounding factors also need to be evaluated.

A majority of participating pregnant women were between age-group 20 - 29 years. However, there were fewer participants between age-groups 15 - 19 years and 40 - 49 years respectively probably because the former age-group comprise

of girls who are pursuing formal education and are less likely to get involved in sexual activities or are probably using safe sex practices while the latter consists of women who have probably obtained the desired number of children, and as a result, are practicing family planning to prevent unwanted pregnancies as suggested by the KDHS [21] data.

High prevalence of *E. coli* in pregnant women between 30 - 39 years was reported in the present study. The reason could be due to the fact that many women within this age group are likely to have had many children before the present pregnancy and it has been reported that multigravida is a risk factor for acquiring *E. coli* in pregnancy [15]. However, certain contraceptive methods are also said to increase the risk of contracting an infection, although women are most sexually active at this age [20]. Furthermore, this could be due to lower pH compared to the genital track of younger women whose genital tracks are acidic, which is inimical to the growth of bacterial organisms including *E. coli*. However, the scope of our protocol was limited to the selected pregnant outpatient participants whose physiological conditions were not investigated.

The current study showed that progesterone levels significantly increased during third trimester and the most prevalent bacteria associated with this increase was *E. coli* even though age and increase in progesterone levels had no significant association with *E. coli* infection. Further research is required to identify bacterial infections among the neonates in hospitals to determine the transmission dynamics of these infections. The study by Barcaite *et al.* [22] found that mothers who had *E. coli* bacterial infections were also infected by group B *Streptococcus*, which is a major cause of fatal neonatal sepsis, although these deaths could easily be controlled by screening and treatment of infected women during pregnancy. It is therefore recommended that pregnant women should routinely be diagnosed and treated of uropathogens to improve maternal and neonate health.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviation

UTI	Urinal Tract infection;
CFU	Colony Forming Unit;
CLED	Cystine lactose Electrolyte Deficient Agar;
MTRH	Moi Teaching and Referral Hospital;
ELISA	Enzyme-Linked ImmunoSorbent Assay;
Spp	Species.