Reference Intervals for Serum Biochemistry Analytes for Pregnant Mothers of Taita-Taveta County, Kenya

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Abstract*: - Reference intervals for pregnant women for blood analytes which are known to change with the trimester of pregnancy are rare. Most clinical laboratories in Africa including Kenya use reference intervals for non-pregnant women developed using western populations to interpret laboratory results for pregnant women which is inappropriate; important pathological changes may be missed, and normal changes may be interpreted as pathological conditions. The aim of this study was to develop trimester specific reference intervals for fifteen serum biochemistry analytes for pregnant women of Taita-Taveta County, Kenya. This was a cross-sectional study involving 296 healthy pregnant women randomly recruited in their second and third trimester attending Moi Subcounty Hospital antenatal clinics from the 16th week after meeting the inclusion criteria, between May 2015 and December 2017. Five millilitres of venous blood was drawn from each participant into plain vacutainer tubes, allowed to clot and then centrifuged to obtain serum. The levels of the serum biochemistry analytes were measured using Clinical Chemistry Autoanalyzer (Integra 400) and reported using SI units. Reference intervals spanning the 2.5 and 97.5 percentiles of each of these analytes were calculated using Clinical Laboratory Standards Institute (CLSI, 2010) guidelines on the obtained non-parametric dataset. Trimester independent reference intervals for total protein, albumin, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, blood urea nitrogen, potassium, chloride, and calcium were established. Trimester dependent reference intervals for alkaline phosphatase, total bilirubin, direct bilirubin, creatinine, uric acid, and sodium were established. In conclusion, trimester specific reference intervals were developed for serum biochemistry analytes for pregnant women of Taita-Taveta County, Kenya different from those reported in literature. These developed reference intervals can be adopted for accurate diagnosis of pathological conditions during pregnancy for this population.

Key words: **pregnant mothers, Trimester, Serum Biochemistry, Reference intervals, Taita Taveta, Kenya.**

I. INTRODUCTION

Reference intervals for serum biochemistry analytes for pregnant women are known to physiologically and biochemically change through the three trimesters of pregnancy and differ from those of non-pregnant women. Alterations in serum biochemistry analytes in the three trimesters of pregnancy have been reported for albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, total bilirubin, creatinine, blood urea nitrogen, uric acid and calcium (8,6,1). These include the first trimester which covers the last menstrual period to the 13th week, the second trimester which covers the period between the 14th-27th week, and the third trimester which covers the period between the 28th-42nd weeks. However, most clinical laboratories in most African countries including Kenya use reference intervals developed by the western countries (mostly from Europe and North America) for non-pregnant women to interpret clinical laboratory results for pregnant mothers which is inappropriate. Use of inappropriate reference intervals increases the risk of misdiagnosis of an important pathological condition which may be detrimental to the mother or fetus, and erroneously interpreting normal condition as a pathological condition which may lead to initiation of therapy for non-existing disease condition. The pathological conditions encountered during pregnancy include pre-eclampsia, hemolysis, increased liver enzymes activities and reduced levels of platelets, blood clotting disorders, and intrauterine infections which may lead to premature delivery or intercurrent diseases like diabetes and appendicitis. It is known that these serum biochemistry analytes for pregnant mothers may be affected by factors such as nutritional status, geographical location, ethnicity, genetics, the instrument and reagents used in analyte estimation, inclusion criteria, among others. For example, western Kenya (8) reference intervals for pregnant women for some serum biochemistry analytes differ from those of the Denmark (6) and American (1) populations. This led to the International Federation of Clinical Chemistry to recommend that every clinical laboratory should develop its own reference intervals using the local population. The use of the correct reference intervals for serum biochemistry analytes for pregnant women would lead to improved care and safe lives and money because to improved diagnosis. The aim of this study was therefore to develop reference intervals for liver and kidney function tests, and electrolytes for pregnant women of Taita-Taveta County, Kenya.

II. MATERIALS AND METHODS

Study site

This study was carried out at the Department of Clinical Chemistry, Moi Subcounty Hospital, Voi, Kenya between May 2015 and December 2017.

Study design

This was a cross-sectional study involving fifteen routinely requested for serum biochemistry analytes from normal pregnant mothers randomly recruited in their second trimester when they commenced attending their antenatal clinics in the 16^{th} week. To minimize bias, at least ten pregnant mothers were recruited weekly.

Study population

The 296 healthy pregnant women, comprised of 124 in their second trimester and 172 in their third trimester who had registered to attend Moi Subcounty Hospital antenatal clinics after meeting the inclusion criteria. The gestational age of each pregnant mother was estimated through an ultrasound scan, and the expected day of delivery recorded in the antenatal clinic booklet.

Inclusion and exclusion criteria

The study included pregnant women who met the following criteria: those who consented to participate by signing a consent form, pregnant women in their second and third trimester with no history of smoking, alcohol consumption, high blood pressure, diabetes mellitus, and no previous history of taking parallel medications like antituberculosis, and antiretroviral drugs. Pregnant mothers on anti-malarial and mineral supplements were also included in the study. The study excluded pregnant women who did not consent to participate in the study, pregnant women who were smoking, hypertensive, and alcoholics. Also excluded were pregnant women with diabetes mellitus, malaria, syphilis, HIV/AIDS, and hepatitis B and C. Absence of syphilis, HIV/AIDS, and hepatitis B and C were confirmed by running serum samples in the Clinical Laboratory, while the absence of other diseases was obtained through physical and clinical examination and history by a physician, and self-reporting using a questionnaire.

Collection of blood samples for routine serum biochemistry analytes

The 296 healthy pregnant women, comprising 124 in their second trimester and 172 in their third trimester were recruited from women attending antenatal clinics for care at Moi Subcounty Hospital, Voi, after meeting the inclusion criteria between May 2015 and December 2017. Five millilitres of venous blood was drawn with a five milliters syringe from each of the 296 pregnant women into plain vacutainer tubes which were correctly labeled with the study number and participants name, allowed to clot, and the centrifuged at 3000 g for five minutes. Serum was separated with a Pasteur pipette for each specimen and transferred into two (duplicate) vials on which a bar cord was used for identification purposes. Measurements were performed for the fifteen serum biochemistry analytes using Clinical Chemistry Autoanalyzer (Integra 400) and reported using SI units.

Estimation of the level of serum biochemistry analytes

Serum total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bilirubin (T-BIL), direct bilirubin (D-BIL), creatinine (CREAT), blood urea nitrogen (BUN), uric acid (UA), potassium (ion selective electrode) (K), sodium (ion selective electrode) (NA), chloride (ion selective electrode) (CL), and calcium (ion selective electrode) (CA) measurements were performed using a well-calibrated, quality controlled Clinical Chemistry Autoanalyzer (Integra 400) and reported using SI units.

Quality control (QC)

Two multisera, normal (PNU) and pathological (PPU) were used for the quality control for the analytical process during the study period. The QC multisera were either ready to use or in lyophilized form. Those that were lyophilized were reconstituted as per manufacturer's instructions. The prepared quality control multisera were used to perform internal quality control assessment or any other time the study procedures were being undertaken (Bolann and Omenås, 1997).

Statistical analysis

For each participant, data for the two trimesters was initially recorded into a laboratory notebook, then entered into the Excel spreadsheet and cleaned. The clean data was exported to SPSS software version 20 for statistical analysis. Data was initially subjected to normality tests including mean, median, mode, skewness and kurtosis. Since the normality tests indicated that the data was non-parametric, the data was expressed in terms of median and range (Table 1). Statistical comparisons of the value of each of the measured analytes for the two trimesters was carried out using Mann-Whitney U Test.

Ethical approval

This study was approved by Kenyatta University Ethical Committee Ref Number I84/31987/15/ NACOSTI Ref number 16/22096/14531, Taita-Taveta county Medical director.

III. RESULTS

Internal quality control for liver and kidney function tests and electrolytes of this study

Two quality control products were used to verify that the instrument used in analyzing the fifteen serum biochemistry analytes is operating within the predefined specifications and therefore producing accurate and reliable results. These were the normal (PNU) and pathological (PPU) quality control

materials. The results of these internal quality controls for the fifteen liver and kidney function tests and electrolytes were all within the predefined specifications indicating that the Clinical Chemistry Autoanalyser (Integra 400) was releasing accurate and reliable results (Table 1).

Table 1: Internal quality control for liver and kidney function tests and electrolytes of this study

Analyte (unit)	QC Type	Assigned QC Report			Study QC Report		
		Mean	SD	% CV	Mean	SD	% CV
TP (g/L)	PPU	46	2.00	4.3	46.6	2.3	2.5
	PNU	68	3.40	5.07	67.5	3.4	5.03
ALB (g/L)	PPU	29.6	2.0	6.07	46.0	2.3	5.00
	PNU	48.8	2.0	4.1	102	5.0	4.90
ALT (U/L)	PPU	139	7.0	5.04	143.1	2.5	1.75
	PNU	51	3.0	5.88	49.3	2.0	4.09
AST (U/L)	PPU	122	6.0	4.92	145.5	1.9	1.32
	PNU	38	2.0	5.26	44.8	1.3	2.99
ALP (U/L)	PPU	259	13.0	5.0	226.8	5.1	2.23
	PNU	102	5.0	4.9	83.8	2.6	3.16
GGT (U/L)	PPU	259	13	5.02	264	10	3.77
	PNU	53	3.0	5.66	53.0	3.0	6.30
T-BIL (µmol/L)	PPU	66.3	4.9	7.39	93.5	3.1	3.27
	PNU	17.1	1.0	5.85	21.7	0.9	4.26
D-BIL (µmol/L)	PPU	33.7	2.5	7.42	36.32	0.75	2.06
	PNU	12.7	1.9	14.96	8.52	0.29	3.35
CREAT (µmol/L)	PPU	398	20	5.03	422	20	4.65
	PNU	92	5	5.43	93	5	5.56
BUN (mmol/L)	PPU	26.3	1.3	4.94	22.5	1.9	8.31
	PNU	7.4	0.4	5.4	7.0	0.3	4.83
UA (µmol/L)	PPU	628	28	4.46	605	18	3.4
	PNU	276	12	4.35	265	15	5.77
K (mmol/L)	PPU	5.2	0.6	3.0	6.5	0.1	2.0
	PNU	5.2	0.6	3.0	4	0.6	1.5
NA (mmol/L)	PPU	144	4.0	2.8	144	2.5	1.7
	PNU	124.3	2.1	1.7	129	5	3.87
CL (mmol/L)	PPU	116	3.0	2.6	115.8	2.9	2.5
	PNU	85.3	2.6	3.1	105	5	4.76
CA (mmol/L)	PPU	3.26	0.12	3.77	**0.0**	**0.0**	**0.0**
	PNU	2.12	0.08	3.9	**0.0**	**0.0**	**0.0**

Normality of liver and kidney tests and electrolytes for pregnant mothers of Taita-Taveta County, Kenya

The normality of the dataset generated by the Clinical Chemistry Autoanalyser (Integra 400) of the fifteen liver and kidney tests and electrolytes for second and third trimester of pregnant mothers of Taita-Taveta County, Kenya was investigated by calculating the mean, median, mode, skewness and kurtosis. Results indicate that the dataset for all the fifteen serum biochemistry analytes were not normally distributed; they were therefore expressed as median and range and statistically compared using Mann-Whitney U test (Table 2).

Table 2: Normality of liver and kidney tests and electrolytes for pregnant mothers of Taita-Taveta County, Kenya

Analyte (unit)	Statistics				
	Mean	Median	Mode	Skewness	Kurtosis
TP (g/L)	66.99	67.60	70.00	-3.433	28.614
ALB (g/L)	38.30	38.50	37.50	**-0.645**	2.195
ALT (U/L)	13.03	10.60	10.00	11.177	159.10
AST (U/L)	20.25	18.00	16.00	8.645	111.219
ALP (U/L)	17.04	2.10	0.00	4.508	25.292
GGT (U/L)	16.44	9.10	6.00	3.306	13.241
T-BIL (µmol/L)	4.55	2.60	1.60	8.515	77.736
D-BIL (µmol/L)	1.15	0.80	0.60	3.217	16.608
CREAT (µmol/L)	55.08	51.00	50.00	1.333	3.144
BUN (mmol/L)	1.97	1.86	2.00	1.594	4.998
UA (µmol/L)	0.21	0.21	0.21	13.795	221.052
K (mmol/L)	4.37	4.32	4.00	**0.240**	**-0.517**
NA (mmol/L)	132.6	132.8	133.2	-7.002	71.155
CL (mmol/L)	98.70	99.50	95.50	-5.281	41.230
CA (mmol/L)	1.70	2.00	2.00	-1.695	1.547

Established reference intervals for liver and kidney tests, and electrolytes for pregnant mothers of Taita-Taveta County, Kenya

The established reference intervals for liver and kidney tests, and electrolytes for expectant mothers of Taita-Taveta County, Kenya are presented in Table 3. Results indicate that, the established reference intervals for liver and kidney tests, and electrolytes for pregnant mothers of Taita-Taveta County, Kenya in their second trimester for TP, ALB, ALT, AST, GGT, BUN, K, CL, and CA were similar to those for pregnant mothers in their third trimester ($\rho > 0.05$); hence combined reference intervals for these parameters were established. The combined (second & third trimester) established reference intervals for liver and kidney tests, and electrolytes for pregnant mothers of Taita-Taveta County, Kenya for TP is 67.6 (55.6-72.2) g/L, ALB is 38.5 (29.1-46.7) g/L, ALT is 10.6 (3.8-42.4) U/L, AST is 18 (9.7-41.9) U/L, GGT is 9.1 (1.7-79.9) U/L, BUN is 1.8 (0.8-4.1) mmol/L, K is 4.3 (3.6-5.2) mmol/L, CL is 99.5 (89.9-105.7) mmol/L, and CA is 2 (0.1-2.1) mmol/L. Results also indicate that, the established reference intervals for liver and kidney tests, and electrolytes for pregnant mothers of Taita-Taveta County, Kenya in their second trimester for ALP, T-BIL, D-BIL, CREAT, UA, and NA significantly differed from those of pregnant mothers in their third trimester of the same County ($\rho < 0.05$). The established reference interval for liver and kidney tests, and electrolytes for these pregnant mothers of Taita Taveta County, Kenya for ALP is 3.1 (0-133.9) U/L in their second trimester and 1.9 (0-165.8) U/L in the third trimester, T-BIL is 2.4 (0-14.5) µmol/L in their second trimester and 3.5 (0.4-17) µmol/L in their third trimester, D-BIL is 0.9 (0-3.1) µmol/L in their second trimester and 0.8 (0.1-6.5) µmol/L in their third trimester, CREAT is 48 (29.6-113.4) µmol/L in their second trimester and 52.5 (36.3-89) µmol/L in their third trimester, UA is 0.22 (0.18-0.35) µmol/L in their second trimester and 0.21 (0.15-0.32) µmol/L in their third trimester, and NA is 133.7 (126.3-140.6) mmol/L in their second trimester and 132 (121.9-139.9) mmol/L in their third trimester (Table 3).

Table 3: Established reference intervals for liver and kidney tests, and electrolytes for expectant mothers population of Taita-Taveta County, Kenya

Analyte (unit)	Trimester	N		Percentile		Reference Interval	IV	Difference between second and third trimisters	
			Median	2.5^{th}	97.5^{th}			Z value	Sig
TP (g/L)	2&3	**296**	**67.6**	**55.6**	**72.2**	**55.6-72.2**	**16.6**	0.667	$\rho = 0.5048$
	2	124	67.3	48.2	76.5	48.2-76.5	28.3		
	3	172	67.7	58.2	76.2	58.2-76.2	18.0		
ALB	2&3	**296**	**38.5**	**29.1**	**46.7**	**29.1-46.7**	**17.6**	1.493	$\rho = 0.1353$

(g/L)	2	124	38.8	27.7	46.9	27.7-46.9	19.2		
	3	172	38.1	31.9	46	31.9-46.0	14.1		
ALT (U/L)	2&3	**296**	**10.6**	**3.8**	**42.4**	**3.8-42.4**	**37.8**	1.229	ρ = 0.219
	2	172	11	4.6	30.9	4.6-30.9	26.3		
	3	124	10	3.3	52	3.3-52	48.7		
AST (U/L)	2&3	**296**	**18**	**9.7**	**41.9**	**9.7-41.9**	**32.2**	0.886	ρ = 0.376
	2	172	18.3	10.3	42.6	10.3-42.6	32.3		
	3	125	21.9	9.31	91.2	9.3-91.2	81.9		
ALP (U/L)	2&3	296	2.1	0	126.3	0-126.3	126.3	2.148	**ρ = 0.031**
	2	**124**	**3.1**	**0**	**133.9**	**0-133.9**	**133.9**		
	3	**172**	**1.9**	**0**	**165.8**	**0-165.8**	**165.8**		
GGT (U/L)	2&3	**296**	**9.1**	**1.7**	**79.9**	**1.7-79.9**	**78.2**	0.750	ρ = 0.657
	2	124	9.1	1.6	115.9	1.6-115.9	114.3		
	3	172	10	1.7	68.5	1.7-68.5	66.8		
T-BIL (µmol/L)	2&3	**296**	2.6	0	14.3	0-14.3	14.3	2.322	**ρ = 0.0202**
	2	**172**	**2.4**	**0**	**14.5**	**0-14.5**	**14.5**		
	3	**124**	**3.5**	**0.4**	**17**	**0.4-17**	**16.6**		
D-BIL (µmol/L)	2&3	296	0.8	0	3.6	0-3.6	3.6	1.690	**ρ = 0.005**
	2	**172**	**0.9**	**0**	**3.1**	**0-3.1**	**3.1**		
	3	**124**	**0.8**	**0.1**	**6.5**	**0.1-6.5**	**6.4**		
CREAT (µmol/L)	2&3	**296**	51	34	95	34-95	61	2.802	**ρ = 0.05**
	2	**124**	**48**	**29.6**	**113.4**	**29.6-113.4**	**83.8**		
	3	**172**	**52.5**	**36.3**	**89**	**36.3-89**	**52.7**		
BUN (mmol/L))	2&3	**296**	**1.86**	**0.8**	**4.1**	**0.8-4.1**	**3.3**	1.341	ρ = 0.1798
	2	124	1.9	0.9	4.3	0.9-4.3	3.4		
	3	172	1.8	0.7	4.1	0.7-4.1	3.4		
UA (µmol/L)	2&3	**296**	0.21	0.16	0.35	0.16-0.35	0.19	3.142	**ρ = 0.0017**
	2	**124**	**0.22**	**0.18**	**0.35**	**0.18-0.35**	0.17		
	3	**172**	**0.21**	**0.15**	**0.32**	**0.15-0.32**	0.17		
K (mmol/L)	2&3	**296**	**4.3**	**3.6**	**5.2**	**3.6-5.2**	**1.6**	1.483	ρ = 0.138
	2	124	4.4	3.6	5.2	3.6-5.2	1.6		
	3	172	4.3	3.6	5.3	3.6-5.3	1.7		
NA (mmol/L)	2&3	296	132.8	126.2	140.5	126.2-140.5	14.3	4.021	**ρ = 0.001**
	2	**124**	**133.7**	**126.3**	**140.6**	**126.3-140.6**	**14.3**		
	3	**172**	**132.0**	**121.9**	**139.9**	**121.9-139.9**	**18.0**		
CL (mmol/L)	2&3	**296**	**99.5**	**89.9**	**105.7**	**89.9-105.7**	**15.8**	0.553	ρ = 0.580
	2	172	99	89.9	104.7	89.9-104.7	14.8		
	3	124	99	89.9	107.9	89.9-107.9	18.0		
CA (mmol/L)	2&3	**296**	**2**	**0.1**	**2.1**	**0.1-2.1**	**2.0**	0.851	ρ = 0.395
	2	124	2	0.1	2.1	0.1-2.1	2.0		
	3	172	2	0.1	2.1	0.1-2.1	2.0		

Results are expressed as Median and range for the number of referent participants in the column labeled N. Statistical comparisons of the median values between second and third trimister referent participants were carried out using Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$.

Comparison of developed reference intervals for liver and kidney tests, and electrolytes for pregnant women of Taita-Taveta County, Kenya with those reported in medical literature

A comparison of developed reference intervals for liver and kidney tests, and electrolytes for pregnant women of Taita-Taveta County, Kenya with those reported in medical literature are presented in Table 4. For the liver function tests, this study's combined lower reference interval limit for TP for pregnant women is similar to that reported by Abbassi-Ghanavati et al. (2009) for Americans, and higher than that of the Denmark population, while the upper limit is higher than that reported for both populations. This study's trimester independent lower and upper reference interval limit for ALB is higher than the trimester dependent reference intervals reported by Abbassi-Ghanavati et al. (2009) for Americans. This study's lower reference interval limit for ALT for pregnant women is higher than that of the western Kenya (8) population, and that reported by Abbassi-Ghanavati et al. (2009) for Americans, but lower than that for the Denmark population, while the upper limit is higher than the trimester dependent limits for western Kenya (8), and Denmark populations and that reported by Abbassi-Ghanavati et al. (2009) for Americans. This study's lower reference interval limit for AST for pregnant women is higher than the trimester dependent limits for western Kenya (8), and Denmark populations, while the upper limit is higher than that of the trimester dependent Denmark population, and lower and higher than that of the western Kenya (8) population in the second and third trimester, respectively. This study's trimester dependent lower reference interval limit for ALP for pregnant women is lower than that of the trimester dependent limits for the Denmark population, and that reported by Abbassi-Ghanavati et al. (2009) for Americans, while the upper limit is lower than that of the trimester dependent Denmark population, and higher and lower than that reported by Abbassi-Ghanavati et al. (2009) for Americans for the second and third trimester, respectively. This study's trimester independent lower reference limit for GGT for pregnant women is higher than the trimester dependent limit reported by Abbassi-Ghanavati et al. (2009) for Americans, while the upper trimester dependent limits are lower. For T-BIL, this study's trimester dependent lower reference interval limit for pregnant women is lower than the trimester dependent limit of the western Kenya (8), and that reported by Abbassi-Ghanavati et al. (2009) for Americans, and the trimester independent limit for the Denmark population, while the upper limit is lower than the trimester dependent limit for western Kenya (8) population, higher than the trimester independent limit for Denmark population, and higher and lower than that reported by Abbassi-Ghanavati et al. (2009) for Americans for the second and third trimester, respectively. For D-BIL, this study's trimester dependent lower reference interval limit is similar to the trimester independent limit reported by Abbassi-Ghanavati et al. (2009) for Americans, while the upper limit is higher.

For the kidney function tests, this study's trimester dependent lower reference interval for CREAT for pregnant mothers is higher than that of western Kenya (8), trimester independent limit for the Denmark populations, and lower and higher than the trimester dependent limits reported by Abbassi-Ghanavati et al. (2009) for Americans for the second and third trimester, respectively, while the upper limit is higher than that of western Kenya (8) and Denmark populations and that reported by Abbassi-Ghanavati et al. (2009) for Americans. For BUN, this study's trimester independent lower reference interval limit is lower than the trimester independent limit for Denmark population and the trimester dependent limits reported by Abbassi-Ghanavati et al. (2009) for Americans, while the upper limit is lower than that of the Denmark population, and lower and similar to that reported by Abbassi-Ghanavati et al. (2009) for Americans for the second and third trimester, respectively. This study's trimester dependent lower reference interval limit for UA for pregnant mothers is lower than that of Denmark population, and that reported by Abbassi-Ghanavati et al. (2009) for Americans, while the upper limit is lower than the trimester dependent limit of the Denmark population, and that reported by Abbassi-Ghanavati et al. (2009) for Americans.

For electrolytes, this study's trimester independent lower reference interval limit is higher than that of the Denmark population and that reported by Abbassi-Ghanavati et al. (2009) for Americans, while the upper limit is higher than that of the Denmark population but similar to those reported by Abbassi-Ghanavati et al. (2009) for Americans. For NA, this study's trimester dependent lower reference interval limit is lower than the trimester independent limit of the Denmark population and that reported by Abbassi-Ghanavati et al. (2009) for Americans, while the upper limit is similar to that of the Denmark population, and lower than that reported by Abbassi-Ghanavati et al. (2009). For CL, this study's trimester independent lower and upper reference interval limits are lower than that reported by Abbassi-Ghanavati et al. (2009) for Americans. For CA, this study's trimester independent lower reference interval limit is lower than that reported by Abbassi-Ghanavati et al. (2009) for Americans, while the upper limit is lower (Table 4).

Table 4: Comparison of developed reference intervals for liver and kidney tests, and electrolytes for pregnant women of Taita-Taveta County, Kenya with those reported in medical literature

Analyte	Trimester	This study RI	Western Kenya population	Denmark population	American population
TP (g/L)	2&3	**55.6-72.2**		30.2-40.5	56.0-59.0
	2	48.2-76.5			
	3	58.2-76.2			
ALB (g/L)	2&3	**29.1-46.7**			
	2	27.7-46.9			26.0-45.0
	3	31.9-46.0			23.0-42.0
ALT (U/L)	2 &3	**3.8-42.4**			
	2	4.6-30.9	0.0-26.0	9.0-32.4	2.0-33.0
	3	3.3-52	0.0-37.0	8.4-36.0	2.0-25.0
AST (U/L)	2&3	**9.7-41.9**			3.0-33.0
	2	10.3-42.6	0.0-39.0	16.2-34.2	
	3	9.3-91.2	0.0-44.0	0.0-40.2	
ALP (U/L)	2 &3	0-126.3			
	2	**0-133.9***		43.8-119.4	25.0-126.0
	3	**0-165.8**		56.4-159.6	38.0-229.0
GGT (U/L)	2&3	**1.7-79.9**			
	2	1.6-115.9			4.0-22.0
	3	1.7-68.5			3.0-26.0
T-BIL (µmol/L)	2 &3	0-14.3		**2-12**	
	2	**0-14.5***	1.4-26.3		1.7-13.7
	3	**0.4-17**	2.2-22.1		1.7-18.8
D-BIL (µmol/L)	2&3	0-3.6			**0-1.7**
	2	**0-3.1***			
	3	**0.1-6.5**			
CREAT (µmol/L)	2 &3	34-95		**42-74**	
	2	**29.6-113.4***	0-59		35-71
	3	**36.3-89**	0-67		35-80
BUN (mmol/L)	2&3	**0.8-4.1**		**1.7-4.3**	
	2	0.9-4.3			1.1-4.6
	3	0.7-4.1			1.1-3.9
UA (µmol/L)	2&3	0.16-0.35			
	2	**0.18-0.35***		**0.71-1.76**	0.85-1.73
	3	**0.15-0.32**		0.74-1.96	1.09-2.23
K (mmol/L)	2&3	**3.6-5.2**		**3.2-4.2**	**3.3-5.1**
	2	3.6-5.2			
	3	3.6-5.3			
NA (mmol/L)	2 &3	126.2-140.5		135-142	129-148
	2	**126.3-140.6***			
	3	**121.9-139.9**			
CL (mmol/L)	2 &3	**89.9-105.7**			**97-109**
	2	89.9-104.7			
	3	89.9-107.9			
CA (mmol/L)	2 &3	**0.1-2.1**			
	2	0.1-2.1			1.1-1.25
	3	0.1-2.1			1.1-1.33

Western Kenya population by Odhiambo et al. (2017), Denmark population by Klajnbard et al. (2010), American population by Abbassi-Ghanavati et al. (2009).

IV. DISCUSSION

Results of this study indicating that the developed reference intervals for liver and kidney tests, and electrolytes for total protein (TP), serum albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), blood urea nitrogen (BUN), potassium (K), chloride (CL), and calcium (CA) for pregnant women in the second trimester are not significantly different from those in the third trimester indicates that these serum biochemistry parameters are trimester independent for this Taita-Taveta County population. These trimester independent reference intervals for pregnant women are 55.6-72.2 g/L for TP, 29.1-46.7 g/L for ALB, 3.8-42.4 U/L for ALT, 9.7-41.9 U/L for AST, 1.7-79.9 U/L for GGT, 0.8-4.1 mmol/L for BUN, 89.9-105.7 mmol/L for CL, 3.6-5.2 mmol/L for K, and 0.1-2.1 mmol/L for CA. Other researchers have also reported trimester independent reference interval limits for CL (1), TP, K (1,6), and BUN (6) which were different from those reported in this study. Cheung and Lafayette (2013) also reported trimester independent K levels in pregnancy. In contrast, other researchers reported trimester dependent reference intervals for ALB and GGT (1), ALT (8, 6,1), AST (8,6), ALP (6,1), BUN and CA (1). These are again different than those observed in the current study confirming the need for each clinical laboratory to develop their own reference intervals using the local population. These differences could be due to factors such as genetics, ethnicity, dietary habits, environment, the methods and reagents used in assessing the levels of the analytes, the comprehensiveness of the inclusion and exclusion criteria, among others. These findings are interesting especially when it is known that the plasma volume and glomerular filtration rate increases during pregnancy resulting in a decrease in most serum constituents. The only serum biochemistry parameters that increase during pregnancy are those associated with the rising levels of oestrogen and progesterone, and placental and bone alkaline phosphatase and uric acid (risk marker for pre-eclampsia) in the third trimester (10).

Results of this study indicating that the developed reference intervals for liver and kidney tests, and electrolytes including alkaline phosphatase (ALP), total bilirubin (T-BIL), direct bilirubin (D-BIL), and creatinine (CREAT) are significantly higher, and sodium (NA), and uric acid (UA) are significantly lower in the second trimester relative to the third trimester, respectively, imply that these biochemical parameters are trimester dependent for this Taita-Taveta pregnant women population. These trimester dependent reference intervals for pregnant women are 0-133.9 U/L ALP in the second trimester and 0-165.8 U/L ALP in the third trimester, 0-14.3 μmol/L T-BIL in the second trimester and 0.4-17.0 μmol/L T-BIL in the third trimester, 0-3.1 μmol/L D-BIL in the second trimester and 0.1-6.5 μmol/L D-BIL in the third trimester, 29.6-113.4 μmol/L CREAT in the second trimester and 36.3-89 μmol/L CREAT in the third trimester, 0.18-0.35 μmol/L UA in the second trimester and 0.15-0.32 μmol/L UA in the third trimester, and 126.3-140.6 mmol/L NA in the second trimester and 121.9-139.9 mmol/L NA in the third trimester. Other researchers also reported trimester dependent intervals for ALP, UA (6, 1), T-BIL, and CREAT (8.1). Further, other investigators reported trimester independent reference intervals for NA (6, 1), T-BIL (6), and D-BIL (1). The significant increase in ALP in the third trimester compared to the second trimester could be due to the increased production of both the placental and bone isoenzyme of this enzyme during the third trimester. The increase in creatinine (CREAT) in the third trimester relative to the second trimester could be due to a decrease in renal plasma flow in the third trimester of pregnancy towards preconception values leading to an increase in serum creatinine (CREAT). The decrease in serum NA levels in the third trimester compared to the second trimester of pregnancy could be due to its increased reabsorption in the proximal nephron due to a large increase in aldosterone and potassium excretion being counterbalanced by increased estrogen and progesterone levels with progesterone acting as a mineral corticoid antagonist in the distal nephron (11). It could also be due to hemodilution caused by vasodilation and secondary increase in water and sodium retention, and increased plasma volume and subsequent antidiuretic hormone (ADH) release during pregnancy (3). Water homeostasis is controlled by thirst and secretion of antidiuretic hormone (ADH) which are regulated by serum osmolality. The decrease in serum uric acid (UA) levels in the third trimester compared to the second trimester could be due to a continuous increase in renal plasma flow and glomerular filtration rate (GFR) and decreases in proximal tubular reabsorption from the first trimester to the third trimester above the preconception value resulting in reduction in serum uric acid (3,7,11). Increase in the level of T-BIL and D-BIL could be attributed to liver damage caused by elevated levels of estrogen during pregnancy towards preconception levels. Pregnancy related increase in estrogen, progesterone and mutations in canalicular bile salts export pump (ABCB11) and multidrug resistant protein-3 (MDR3, ABCB4) gene leads to increased sensitivity to estrogen which impairs sulphation and transport of bile acids, decreases hepatocyte membrane permeability and uptake of bile acid by the liver (5). The raised bile acid concentration interferes with myometrial contractility and induces vasoconstriction of placental chorionic veins, which causes preterm deliveries, meconium staining of liquor, fetal bradycardia, distress and fetal loss correlated with fasting serum bile acid levels higher than 40 μmol/L (5). The differences between the serum biochemistry analytes reference intervals for pregnant women population of Taita-Taveta County and those previously reported in literature from other parts of the world could be due to dietary habits (nutritional status), genetics, health status, environmental, lifestyle including tobacco smoking, alcohol consumption, and sedentary lifestyle. Further, the analytical instruments and reagents used and the selection

criteria of referent pregnant women in different parts of the world may also contribute to the observed differences.

This study had several limitations. One limitation of this study was that it did not include a sample from non-pregnant women from the same referent population to generate baseline data. Another limitation was that it was not possible to recruit pregnant women early enough so as to capture them while in their first trimester. Other limitations included the variation in the number of pregnant women in each trimester due to exclusion of participants during screening, and the time for drawing blood varied daily which may increase the reference intervals due to the circadian rhythms. However, this study generated the first reference intervals for biochemical parameters for pregnant women of Taita-Taveta County population of Kenya to be used locally in the clinical management of women disorders during pregnancy.

In conclusion, the study has developed trimester specific reference intervals for selected serum biochemistry analytes for pregnant mothers in their second and third trimester of Taita-Taveta County which differ from those reported in medical literature. These can be adopted and used to provide cutoff points for diagnosis and management of pregnancy related pathological conditions.

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