Journal of Clinical Chemistry and Laboratory Medicine

OPEN ACCESS Freely available online

Research Article

Establishment of Trimester Based Reference Intervals for Routine Electrolytes among Apparently Healthy Pregnant Women at Debre Markos Comprehensive Specialized Hospital, North West Ethiopia, 2021

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ABSTRACT

Background: Reference interval for electrolytes currently applicable for pregnant women in Ethiopia is used from other populations that are adopted from textbooks and literatures. However, using others` reference interval is might not useful for clinical decisions during pregnancy due to normal changes resulted from different hormonal changes that may affect biochemical parameters.

Objective: The aim of this study was to establish trimester based reference intervals for electrolytes among apparently healthy pregnant women at Debre Markos Comprehensive Specialized Hospital, Northwest Ethiopia.

Methods: Institutional based cross-sectional study was applied and based on the selection criteria, 459 apparently healthy pregnant women were enrolled in this study. Finally, reference intervals were established by non-parametric methods by the recommendation of Clinical and Laboratory Standards Institute guideline C28-A3. The 95% reference interval was estimated by using 2.5th percentile for lower reference limit and 97.5th percentile upper reference limit. The Kruskal Wallis and Post-Hoc tests were applied to compare medians within trimesters and statistically significant difference between trimesters respectively.

Results: The 95% reference interval for biochemical parameters were: Na: 121.7-158.6, 121.85-153, 123.7-149.45, Ca: 1.89-3.25, 1.15-2.92, 1.63-2.97, K: 3.02-6.3, 3.06-6.27, 3.09-5.57, Cl: 94.85-136.9, 94.85-166, 91.55-122, for 1st, 2nd and 3rd trimesters respectively. Besides this, the statistical significant difference within and between trimesters was recorded for almost all parameters.

Conclusion: The reference interval for electrolytes determined in this study was different from the reported reference interval in literature and manufacturer provided as well. Thus, these newly established reference intervals of electrolytes will be valuable for the detection and monitoring of different complications related with pregnancy.

Keywords: Electrolytes; Biochemical profiles; Pregnancy; Reference interval

INTRODUCTION

The Reference Interval (RI) includes the central 95% of distributed values, which is established from apparently healthy individuals [1]. The central values of 95% of the distribution comprises the values between the 2.5th and 97.5th percentiles as recommended by International Standards of Organization (ISO) 15189 and International Federations of Clinical Chemistry (IFCC) [2]. The RI is lower and upper limits of a given biochemical parameter depending on the clinical significance [3].

The RI has different advantages like for disease screening and diagnosis, for monitoring and for progression, for treatment efficacy and guiding patient laboratory test interpretations [4]. It is supposed to establish RI for specific biochemical parameter and biological specimen source in each medical laboratory [5]. Because it helps for accurate interpretation of laboratory data and provide assistance to the clinician in creating more comprehensive clinical perspective for diagnosis and management of patients [6,7]. The reliable and accurate RIs for laboratory analyses are an integral part of the process of correct interpretation of clinical laboratory test results [8].

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Received: 18-Oct-2022, Manuscript No. JCCLM-22-19694; **Editor assigned:** 21-Oct-2022, PreQC No. JCCLM-22-19694 (PQ); **Reviewed:** 04-Nov-2022, QC No. JCCLM-22-19694; **Revised:** 11-Nov-2022, Manuscript No. JCCLM-22-19694 (R); **Published:** 18-Nov-2022, DOI:10.35248/JCCLM.22.05.246

Citation: Tamir W(2022) Establishment of Trimester Based Reference Intervals for Routine Electrolytes among Apparently Healthy Pregnant Women at Debre Markos Comprehensive Specialized Hospital, North West Ethiopia, 2021. J Clin Chem Lab Med.05:246

Studies conducted in Asian and African countries have showed the differences in the RIs compared to the western RIs due to different factors [4,9]. The Clinical Laboratory and Standards Institute (CLSI) also recommend that different factors should be considered during RI establishment. This is due to clinical laboratory parameters are affected by various factors like genetic makeup of individuals and populations, environmental factors like ethnic background, climate, altitude, lifestyles and physiological patterns [10].

Even though the study suggested that pregnancy physiologically may affect biochemical parameters, most laboratory information systems report the electrolytes RI based on non-pregnant women which are not necessarily useful for CDs during pregnancy [11]. This is due to maternal physiology undergoes many changes during pregnancy by the effects of progesterone and estrogen which are produced majorly by the ovary in the 1st trimester and thereafter by the placenta [12]. The increased demand of energy to mother for normal development of growing fetus and adaptations of the maternal body during pregnancy results normal biochemical changes [13,14].

Knowing what changes of parameters are normal during pregnancy is used to diagnose and manage common medical problems of pregnancy related complications like hypertension, gestational diabetes and hypothyroidism [15]. Clinical chemistry parameters like electrolytes play a basic role in distinguishing and understanding of diseased conditions and normal changes in managing adverse health complications [16]. Due to these and other reasons, an international guideline recommends that each country must establish representative RIs for specific groups [17]. Therefore, using locally established RIs which are specific to reference population plays the significant role in the interpretation of laboratory test results and CDs [18,19].

Objectives

General objective: To establish trimester based reference intervals for electrolytes among apparently healthy pregnant women at Debre Markos Comprehensive Specialized Hospital, East Gojam Zone, Ethiopia.

Specific objectives:

- To establish trimester based reference interval for electrolytes among apparently healthy pregnant women (sodium, chloride, calcium and potassium)
- To compare reference intervals of biochemical parameters within trimesters

METHODOLOGY

Study area and period

This cross-sectional study was done on 459 apparently healthy pregnant women at Debre Markos comprehensive specialized hospital. Further, the study lasted for 5 months from February 2021 to June, 2021. Before study initiation, the ethical approval for the study protocol was obtained from the Health Research and Ethics Committee of the Debre Markos University, Debre Markos. Furthermore, informed consent was obtained from each participant and then a structured questionnaire was used to obtain data.

Source and population study population

The source population for this study were all pregnant women attended at Debre Markos Comprehensive Specialized Hospital while, the study population was pregnant women who fulfilled the eligibility criteria during the study period.

Inclusion and exclusion criteria

Apparently healthy pregnant women who had negative serological tests for viral infections; Hepatitis (B,C), HIV and malaria and were attending ANC clinic, at Debre Markos Comprehensive Specialized Hospital during study period were included. While, those pregnant women with known history of any infectious and non-infectious disease like, diabetes mellitus, chronic renal insufficiency, hypertension, heart disease, thyroid disease and liver diseases, chronic alcohol abusers, smokers, viral infections, and who had a history of jaundice within the last 90 days and major surgery within 1 year were excluded from the study.

Sample size and sampling technique

The CLSI recommends both parametric and non-parametric methods to establish RIs. In order to estimate a non-parametric 95% RI, at least 153 reference individuals per trimester [20]. For each trimester, 153 pregnant women were included and 459 apparently healthy pregnant women were selected for the study. Consecutive convenient sampling method was used to get adequate sample size.

Variables of the study

Dependent variable: Electrolytes (Na^+, Cl-, Ca^{++}, K^+)

Independent variables:

1. Gestational ages of pregnancy
   - First trimester
   - Second trimester
   - Third trimester
2. Age, weight, BMI, etc.

Operational and standard definition

Apparently healthy: The absence of disease or, clinical sign and symptom during study period.

First trimester: The gestational period from week 1-12 weeks or 1-3 (in months).

Second trimester: The gestational period from week 13-24 weeks or 3-6 (in months).

Third trimester: The gestational period from week 25-36 and above weeks or 6-9 (in months).

Data collection and laboratory methods

Training was given for data collectors how to approach and interview participants before the actual data collection, about the study participants' rights, objective of the study, confidentiality, procedure of blood sample collection, transportation, storage and measurements. The study subjects those who were eligible for inclusion criteria were contacted when they came to ANC clinic of Debre Markos comprehensive specialized Hospital. The study

subjects who volunteered to give written consent after being told about the merits of the study, related risks were interviewed, and around 5 ml of whole blood sample was collected from each study subjects using clinical chemistry test tubes that contains serum separator. Then centrifugation at 2500 revolution per minute for 5 minutes was applied to separate serum. The Maglumi 800 full-automated clinical chemistry analyzer was employed for the measurement of clinical chemistry parameters.

Laboratory data quality management

To retain the quality of data, the functioning of the clinical chemistry automation was managed by analyzing quality controls before running the samples of study subjects. The quality assurance cycles from pre-analytical to post-analytical phases were controlled based on the standard operating precautions. Every activity like blood sample collection, transportation and laboratory test analysis was based using standard operating procedures to ensure data quality.

The analysis was done in Debre Markos Comprehensive Specialized Hospital of clinical chemistry laboratory under close supervision. The instruments had been calibrated monthly by type-Autocal and the quality controls of both normal and abnormal tests were run daily. The Internal Quality Control (IQC) samples were done by following instructions of the manufacturer beside with the serum sample. The quality of the data that gave reliability and representativeness to the study was maintained by incorporating only the complete data of the patient with in the study period. The collected data was confirmed at different levels for fullness and regularity at the end of every day.

Data processing, analysis and interpretation

The data were rechecked, selected, organized and ordered manually. Then, the data were entered to Epi data version 4.2. and transported to SPSS version 23 for statistical analysis. To get reliable results both parametric and non-parametric analysis were done. The median of each biochemical parameters were calculated using simple descriptive statistics and it was tabulated based on level of trimester. Kruskal Wallis test was used to compare medians parameters within trimesters and the statistically significant difference between trimesters was checked by using Post-Hoc test comparison. The RIs were calculated according to the recommendation of CLSI guideline using percentiles. The 95% RIs was estimated using the lower reference limit at 2.5th percentile and the upper reference limit at 97.5th percentile depending on the clinical significance of the analyst and the p-value<0.05 was measured as statistically significant finding.

RESULTS

Socio-demographic characteristics

About a total of 459 apparently healthy pregnant women were participated in this study. Of these, 401 (87.4%) were from urban and 58 (12.6%) were from rural. The median, minimum and maximum ages of study subjects were 27, 18 and 44 year respectively with range of 26 year.

The distributions of electrolytes

The electrolyte test distribution was found to be with the median values for Na^+ (139,142,142), Ca^{++} (2.36, 2.20 and 2.26) K^+ (3.59, 3.90 and 3.81), Cl- (106,108,107) respective to first, second and third trimesters (Table 1).

Comparison of electrolyte test parameters across trimesters

There was statistically significant difference across groups: Na^+, Ca^{++} and Cl- ($p<0.05$). The electrolyte parameters which is has not statistical significant difference ($p>0.05$) was K^+ (Table 2).

Comparison of routine electrolyte tests RIs with currently in use RIs

The variation was recorded when compared with the RIs of currently in use, manufacturer provided and RIs presented in this study (Table 3).

Table 1: The median (IQR) and 95th RIs of electrolyte tests among apparently healthy pregnant women at Debre Markos Comprehensive Specialized Hospital, North West Ethiopia, 2021.

Test parameter	Trimester	Median (IQR)	95% RI	95% CI	
				Lower limit	Upper limit
Na^+ (mmol/L)	1st trimester	139 (132-143)	121.7-158.6	118.4-125	154.3-163
	2nd trimester	142 (139-144)	121.85-153	120.7-126.34	149-154
	3rd trimester	142 (138-144)	123.7-149.45	120.7-126	148.15-154.15
Ca^{++}(mmol/L)	1st trimester	2.36 (2.19-2.68)	1.89-3.25	1.1-2.07	3.15-3.52
	2nd trimester	2.2 (2.06-2.39)	1.15-2.92	1.09-1.99	2.76-3.21
	3rd trimester	2.26 (2.11-2.50)	1.63-2.97	1.48-1.91	2.89-3.22
K^+(mmol/L)	1st trimester	3.59 (3.45-4.08)	3.02-6.3	2.15-3.15	5.18-14.16
	2nd trimester	3.9 (3.4-4.48)	3.06-6.27	3.04-3.17	5.6-6.46
	3rd trimester	3.81 (3.49-4.25)	3.09-5.57	2.18-3.17	5.21-5.77
Cl-(mmol/L)	1st trimester	106 (102.5-111)	94.85-136.9	92.85-97	124-161
	2nd trimester	108 (105-114)	94.85-166	92.2-97	130.05-168
	3rd trimester	107 (103-110.75)	91.55-122	81.95-96.25	118.3-124

Table 2: Comparison of electrolyte tests by trimester among apparently healthy pregnant women at Debre Markos Comprehensive Specialized Hospital, North West Ethiopia, 2021.

Test parameters	Post-Hoc test		p-value	P*-value
Na^+	1st trimester	2nd trimester	0.031	0.025*
		3rd trimester	0.119	
	2nd trimester	1st trimester	0.031	
		3rd trimester	1	
	3rd trimester	1st trimester	0.119	
		2nd trimester	1	
Ca^{++}	1st trimester	2nd trimester	<0.001	<0.001*
		3rd trimester	0.001	
	2nd trimester	3rd trimester	0.07	
		1st trimester	<0.001	
	3rd trimester	1st trimester	0.001	
		2nd trimester	0.07	
K^+	1st trimester	2nd trimester	1	0.694*
		3rd trimester	1	
	2nd trimester	3rd trimester	1	
		1st trimester	1	
	3rd trimester	1st trimester	1	
		2nd trimester	1	
Cl-	1st trimester	2nd trimester	0.69	0.014*
		3rd trimester	0.265	
	2nd trimester	3rd trimester	0.011	
		1st trimester	0.69	
	3rd trimester	1st trimester	0.265	
		2nd trimester	0.011	
	2nd trimester	0.29-1.00		
	3rd trimester	0.57-1.10		

Note: P* is the average electrolyte value of all the trimesters of healthy pregnant women at Debre Markos Comprehensive Specialized Hospital, North West Ethiopia, 2021.

Table 3: The RIs of electrolyte tests and comparison against RIs of apparently healthy pregnant women and currently in use RIs at Debre Markos Comprehensive Specialized Hospital, North West Ethiopia, 2021.

Test parameter	Trimester	Present study 95% Ris (n=153)	Currently use in Lab RIs	Manufacturer provided RIs
Na^+	1st trimester	121.7-158.6	135-145	135-145
	2nd trimester	121.85-153		
	3rd trimester	123.7-149.45		
Ca^{++}	1st trimester	1.89-3.25	1.15-1.33	1.1-1.4
	2nd trimester	1.15-2.92		
	3rd trimester	1.63-2.97		
K^+	1st trimester	3.02-6.3	3.3-4.9	3.5-5.5
	2nd trimester	3.06-6.27		
	3rd trimester	3.09-5.57		
Cl-	1st trimester	94.85-136.9	98-107	98-108
	2nd trimester	94.85-166		
	3rd trimester	91.55-122		

DISCUSSION

Even though the best way for clinical decision is using appropriate locally established RIs, clinical examination typically begins with collecting sign and symptoms of the patients. Thus, medical laboratories have value if and only if they can be compared the laboratory test results which are far from the usual spread of values found in health and pathological conditions. In clinical practice, electrolytes tests including other organ function tests are commonest laboratory parameters used in day to day patient care [21].

The electrolyte tests are often requested during pregnancy to exclude pregnancy related complications, which may affect health of both mother and the fetus. Diagnostic accuracy should be based on evaluation of results in relation to RVs of the local laboratory [22]. In addition to the differentiation of physiological changes from pathological conditions, the establishment of suitable RIs for pregnant women has the potential to improve diagnostic quality, which could lead to increased survival, reducing unnecessary treatment and cost savings.

In this study, the RIs of serum sodium ions, potassium ions, calcium ions and chloride ions, for apparently healthy pregnant women have been established. Obviously, during pregnancy a raise in serum sodium, potassium, and calcium and chloride levels associated with pregnancy has been observed. This is contradicted with the finding conducted in Sardar Patel Medical College, Bikaner [23]. This might be due to variations in race, geographical locations, lifestyle and genetics of the study populations.

In the present study, all of the values obtained were different from that of RIs of manufacturer provided with the reagents. The difference might be because of the pregnant mother undergoes significant anatomical and physiological changes which have begun after conception that affect every organ system by hormonal actions [24]. These physiological changes are happened to demand the developing of a fetus, maintain homeostasis, and prepare for birth and lactation [25]. Besides this, the variation might be, since the comparison was with RIs non-pregnant women who are from Caucasian populations and who are different in genetics, geographical locations and lifestyles [25].

Regarding the statistical significance, Na^+ ($p=0.025$) between 1st and 2nd, Ca^{++} ($p<0.001$) between 1st and 2nd and 1st and 3rd Cl^- ($p<0.014$) between 2nd and 3rd trimesters. While, the biochemical parameters which have no statistically significant difference ($p>0.05$) is serum potassium (K^+) levels.

CONCLUSION

In conclusion, the RIs established for the electrolyte tests in this study were different from currently in use and manufacturer provided RIs. The established RIs for each parameter were different among trimesters. Thus, patient management and interpretation of laboratory findings of the population should be based on the locally derived RIs, which are reference population specific. Conducting similar national wide study to determine the biochemical RIs of the Ethiopian population using comparison groups as a whole is very essential.

LIMITATION OF THE STUDY

Even though, this finding meets the minimum CLSI requirements for setting RIs, it did not include a parallel sample of non-pregnant from the similar reference population.

FUNDING

This work was supported by Debre Markos University, Ethiopia. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ETHICS STATEMENT

Theethicalclearance was obtained from theethicalreview committee of Debre Markos University, College of Health Sciences. A formal letter was submitted to Debre Markos Comprehensive Specialized Hospital and permission was assured. Written informed consent was signed before sample collection. And after they agreed and signed on the consent form blood sample was collected on clinical chemistry test tube for laboratory analysis. And laboratory test for each parameter was performed by following standard operating procedures of clinical chemistry.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that there is no competing interest.

AUTHORS' CONTRIBUTIONS

WT has conceived and designed the study protocol, statistical analysis, interpretation of the data, and developing the initial drafts of the manuscript.

ACKNOWLEDGMENTS

The author acknowledges Debre Markos University College of Health Sciences, Debre Markos Comprehensive Specialized Hospital, data collectors and study participants.

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