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Serum Total and Placental Alkaline Phosphatas at Second and Third Trimesters of Normal Pregnant Mothers from July to Nov 2015 at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Serkalem Hailu entitled:

``Serum Total and Placental Alkaline Phosphatase at Second and Third Trimesters of Normal Pregnant Mothers from July to Nov 2015at Zewditu Memorial Hospital, Addis Ababa, Ethiopia: A Hospital based Cross Sectional Study `` and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Clinical Laboratory Sciences (Clinical Chemistry Specialty Track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of Abbreviations

BP	Blood Pressure
DNA	Deoxyribonucleic Acid
DGKC	German Clinical Chemistry association
EC	Enzyme Commission Number
HSAP	Heat- Stable Alkaline Phosphatase
IFCC	International Federation of Clinical Chemistry
IgG	Immunoglobulin G
IUL	International Units per Liter
4-NPP	4-Nitrophenylphosphate
P	Probability
PLAP	Placental alkaline Phosphatase
QC	Quality Control
SCE	Scandinvian Committee on Enzymes
SPSS	Statistical Package for Social Sciences
TALP	Total Alkaline phosphatase
ANC	Antenatal Clinic
ZMH	Zewditu Memorial Hospital

Operational Definitions

Ping-pong catalytic mechanism: is also called a double displacement reaction and it means that one or more products are released before all substrates bind the enzyme

Km values: is an affinity of an enzyme towards substrate

Gestational age: is how far along the pregnancy is. It is measured in weeks, from the first day of the woman`s last menstrual cycle to the current date.

Trimester: is a period of three months when women in pregnant; 13-28 week second trimester, 29-40 week third trimester

Hypertensive disorder: is defined as group of diseases which includes: preeclampsia, eclampsia, gestational hypertension and chronic hypertension. The systolic blood pressure ≥140 or diastolic blood pressure ≥90mmHg

Quality control: is defined as a process to identify errors in a procedure. QC`s sera contains the required levels of an analyte to validate each test result derived from the analyzer, over its clinically significant ranges.

Between-day precision: is an experiment conducted over a period of twenty days to provide more realistic estimate of the variation that will be seen in samples over time, also called "day-to-day" or "total" imprecision of the method

Westgard rules: are commonly used to analyze data in Levey-Jennings chart

Parity: is defined as the number of times that she has given birth to a fetus with a gestational age of 28 weeks or more, regardless of whether the child was born alive or was stillborn

Primiparity: is a woman who is pregnant for the first time

Multiparity: is a women who has given birth more than once

Grand multiparity: is a woman who has delivered five or more infants

Abortion: means termination of pregnancy; women who are up to 20 weeks pregnant

ABO system: is a classification system for human blood that identifies four major blood type based on the presence or absence of two antigens, A and B on red blood cell

Abstract

Background: Alkaline phosphatase (ALP) (EC 3.1.3.1.) is an enzyme which catalyzes the hydrolysis of phosphate esters at an alkaline PH. Placental alkaline phosphatase isoenzyme (PLAP) has several vital roles during pregnancy for supporting fatal growth and development. The change of PLAP may be associated with high blood pressure, preterm delivery and other complication. It could be an indicator of overall placental performance and pregnancy outcomes. Therefore, there is the need to determine and proper interpretation in our local setup.

Objectives: To evaluate serum total and placental alkaline phosphatase at second and third trimesters in normal pregnant mothers in our local setup.

Method: Across-sectional study was done on randomly selected 333 consenting apparently healthy pregnant mothers at Zewditu Memorial Hospital in Addis Ababa, Ethiopia. Maternal demographic characteristic was obtained through questionnaires and medical chart review. The TALP and PLAP were analyzed by Human 4-Nitrophenylphosphatas (4-NPP) substrate. The data was analyzed using the SPSS software, version 21. The data was assessed using non-parametric analysis; hence the data was found to be skewed. $P< 0.05$ was considered statistically significant.

Result: The median of TALP and PLAP in second trimester were (163 IU/L) and (25 IU/L) respectively while in third trimester were (334 IU/L) and (141IU/L) respectively and at term were (449 IU/L) and (230 IU/L) respectively ($P< 0001$). Strong positive correlation observed between TALP ($r^2 = 0.805$, $p<0.001$) and PALP with gestational age ($r^2 = 0.805$, $P< 0.001$). Chi-square showed positive association with blood group and blood pressure ($P<0.05$).

Conclusion and Recommendation

There was significant increasing serum TALP and PLAP with advancing gestational age. There was a peak levels at term. Strong positive correlation observed between TALP and PALP with gestational age. Also, blood group and blood pressure were significantly associated. Thus, it could be used as an easy accessible and affordable biomarker for monitoring the status of pregnancy.

Keywords: Total and Placental alkaline phospatase, pregnant mothers Trimester

1. Introduction

1.1. Background

Alkaline phosphatase (ALP) (EC 3.1.3.1.) is an enzyme which catalyzes the hydrolysis of phosphate esters at an alkaline PH. Alkaline phosphatase is present in many tissues of the body with especially high level in the liver, bone, placenta and intestine.ALP derived from different tissues share catalytic properties, but differ in their chemical and physical properties which include difference in relative rates of reaction with various substrate, response to the presence of selected inhibitor, stability to denaturation by heat, electrophoretic mobility and immunological characteristic. In normal pregnancy the serum level of total alkaline phosphatase increases 2-to 3-fold the upper limit of the reference range from non-pregnant women due to additional production of placental alkaline phosphatase (PLAP). It becomes a major ALP in the maternal blood circulation at term and persists in the serum up to 12 weeks after delivery (1-2).

Several researches have been reported on PALP isoenzyme may has vital role during pregnancy for supporting fetal growth and development such as DNA synthesis and cell proliferation of the human fetal fibroblasts (3). Also, it acts as a receptor for IgG to transport transplacentaly from mother to the fetus. Thus, it shows that PALP isoenzyme helps to provide passive immunity to the fetus during gestation (4).In addition PALP isoenzyme involves nutrient mobilization through transplacentaly from mother to fetus. Increasing production has been associated with the nutritional demand of the fetus (5).

Increasing or decreasing of PALP isoenzyme in maternal serum may be associated with preterm delivery, toxemia of placenta, low birth weight, placenta insufficiency, intrauterine growth retardation, premature rupture of membranes and so on. So, it could be an indicator of overall placental performance and pregnancy outcomes (6-8).

This suggests that serum PLAP isoenzyme could be used as a biomarker to be integrated into the routine laboratory to monitor the status of pregnancy at its specific trimester. Proper diagnosis and interpretation of it can lead to proper managements of pregnancy outcome and may reduce pregnancy complications in both mother and fetus. Therefore, this study was aimed to evaluate

serum TALP and PLAP at second and third trimester in normal pregnant mothers in our local setup.

1.1.1. Structure and biochemistry of alkaline phosphatase

Alkaline Phosphatase is dimmeric enzymes that contain three metal ions in each catalytic site, i.e., two Zn and one Mg. The enzymes catalyze the hydrolysis of monoesters of phosphoric acid and also catalyze a transphosphorylation reaction in the presence of large concentrations of phosphate acceptors which release of inorganic phosphate and alcohol at alkaline pH (10.3). It involves the activation of a serine by a zinc atom. ALPs have higher specific activity and Km values s; display lower heat stability; are membrane-bound and are inhibited by L-amino acids and peptides through an uncompetitive mechanism (9).

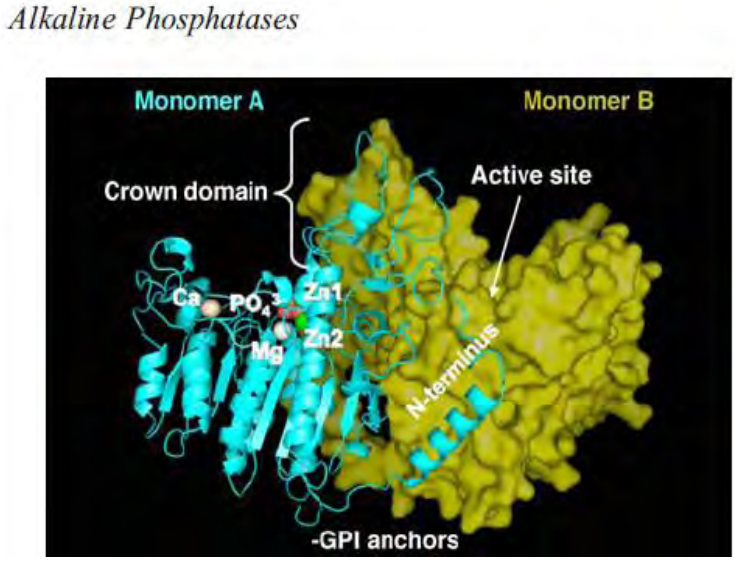

Figure 1.Three-dimensional structure of APL. Monomer A is shown in ribbon representation and in cyan, while monomer B is shown in surface representation in yellow. Indicated are the active site metals, Zn1, Zn2 and Mg, the novel fourth metal site occupied by Ca, the crown domain and the amino terminal arm (9).

1.1.2. Catalytic mechanism of alkaline phosphatase

It is ``ping-pong`` catalytic mechanism. It involves the activation of a serine by a zinc atom. The tetrahedral phosphate participates in interaction with the two active site zinc ions. Mg bound water molecule (10).

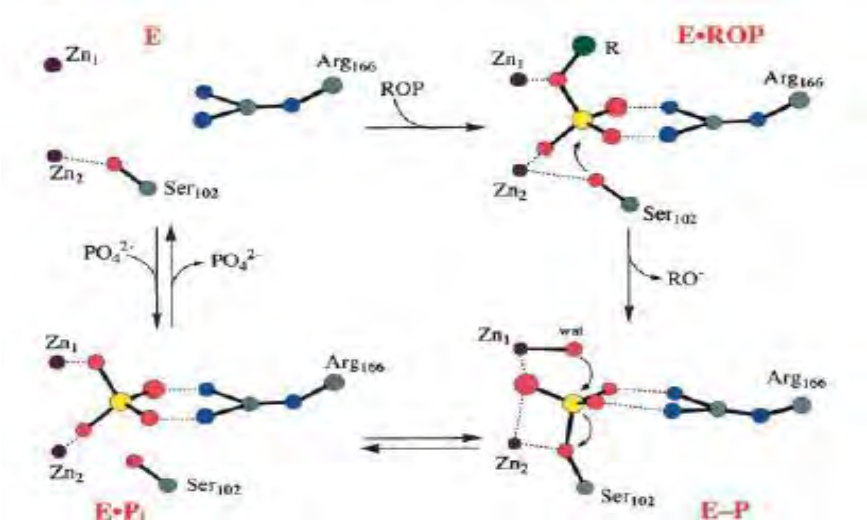

Figure2.Mechanism of the alkaline phosphatase reaction.It is ``ping-pong`` mechanism based on the x-ray crystal structure of the enzyme with inorganic phosphate bound in the active site (10).

1.1.3. Separation methods of alkaline phosphatase isoenzyme

Stability of denatureation to heat; this method is based on one of difference in isoenzymes deactivation rate at different temperatures. Implementation of heat inactivation method requires a very precise control of experimental conditions such as temperature and duration of incubation. Bone and liver ALP activities inactivate by heat but TALP isoenzyme activity stable.

Chemical inhibition; the intestinal and placental ALP isoenzyme strong inhibition by L-phenylalanine but bone isoenzyme strong inhibition by urea.

Immunological Techniques; provide the best quantitative measurements of placental and intestinal ALP by using polyclonal or monoclonal antisera. Much more difficult is the production of antibodies that selectively react with different products of the tissue non-specific ALP gene, including liver and bone isoforms, as these antibodies should recognize specific sugar side chains.

Electrophoresis mobility; In gel electrophoresis, isoenzyme fragments are drawn through a thick gel by an electric charge. Each isoenzyme has a distinct charge of its own because of its unique amino acid sequence. This enables gel electrophoresis to separate the fragments into bands for identification. ALP zones are made visible by incubating the gel in a solution of

buffered substrata. The liver ALP moves rapidly toward the anode following bone ALP then intestinal ALP migrates slowly than the bone ALP, whereas the placental ALP appears as a discrete band overlap the diffuse bone fraction (1, 11).

1.2. Statements of the problem

Understanding the clinical characteristic and possible maternal factors that associated with normal and abnormal pregnancy remains a major goal of obstetrics. It is impossible to manage the status of pregnancy and also to take intervention without such knowledge. Also, during normal pregnancy the levels can be misinterpreted as abnormal (2). Numerous studies have demonstrated the levels of serum TALP and PALP have a series association with the status of pregnancy and pregnancy related complication.

Pregnancy associated hypertension remains unsolved significant problem in obstetrics even though a number of research had been done. In hypertensive pregnant women at term the PLAP has been reported 55% from TALP. Early detection of hypertensive disorder using HSAP parameter was significantly decreased the number of morbidity and mortality. Hence, its measurement is an easy accessible and affordable in our environment (12).

Prevention of preterm deliveries should be a priority for obstetrics, prematurity remains the main cause of complications and mortality in newborns. Diagnosis was made after hospitalization and complications. A study has shown the mean value of HSAP was 1280 IU/l within the first eight weeks of pregnancy. The probability of preterm delivery where HSAP value is >2, is 12.2 times greater than term delivery. Therefore, it can be used as early markers for predicting preterm delivery developing later in pregnancy (13).

In placental insufficiency with low birth weight the study shown serum TALP elevated up to 10.5-fold; of this 94.05% was placental isoenzyme had seen in the 3rdtrimester of gestation.(14). Also, loss of syncytial membranes in immature villi led to increased TALP up to 3609 IU/L at term; of this 96% was PLAP concentrations in the maternal circulation and decreased ALP in the placenta. Their finding shed new light on a significant change in placental function (15).

According to above previous studies TALP and PLAP isoenzyme levels in maternal serum have a series association with the status of pregnancy and pregnancy related complication. Therefore,

the good understandings of those associations are crucial. It may be support to clinicians for allow for earlier intervention. These studies propose that it may be an early biomarker for the diagnosis of the status of pregnancy.

For developing countries such as Ethiopia the utilization of this parameter is not questionable, especially in lower and middle level health facilities without need of others advanced diagnostic tools since, it is easy accessible and affordable tests in our environment. However, currently as to our knowledge it is not practiced as one of the routine clinical tests for monitoring the status of pregnancy. Therefore, there is the need to determine and proper interpretation of serum TALP and PLAP at second and third trimesters in normal pregnant mothers in our local setup.

1.3. Literature Review

The pregnant mother experiences physiological changes to support fetal growth and development during pregnancy. Sometime these changes might be perceived as abnormal. Also, the clinical characteristic and possible maternal factor affect normal pregnancy.

A prospective study was conducted in France on liver function tests in normal pregnancy in 103 pregnant women with a non-pregnant woman (control group). Among the 103 pregnant women, 34 were first trimester, 36 second trimester, and 33 were third trimester. Fasting blood samples were taken. Serum TALP activity was measured at 30 ^{0}C using the method of Bessey et al. Upper limits of normal values in this laboratory for non-pregnant women are 90 IU/L. The result was significantly higher in the third trimester in normal pregnant women compared with non-pregnant women and during the second trimester compared with the first trimester .The identification of these physiological changes is important for the diagnosis of liver diseases during pregnancy (16).

In Northern Nigeria cross sectional descriptive study was done to determine pattern serum TALP activity in normal third trimester pregnancy among 100 healthy pregnant women. Serum TALP activity was measured at 37 ^{0}c using the 4-Nitrophenylphosphate (4-NPP) method, which is in use in the ABUTH chemical pathology laboratory. The serum TALP activity was found to be higher when compared to the normal population, with a gradual increase with advancing gestational age. Subjects in the gestational age group of 28 to 30 weeks had a serum TALP

activity of 46-138 IU/L, with a continuous increase of up to 213 IU/L at 39 weeks of gestation. A sudden drop in activity was observed at the gestational age of 40 weeks and above. They have been confirmed serum TALP activity in normal 3rd trimester pregnancy was found to be (41-206 IU/L) for this environment (17).

A cross sectional study was conducted in UK on the effect of gestational age on levels of serum alkaline phosphatase isoenzymes in healthy pregnant women. This study was carried out in 67 normal pregnant women compared with 18 normal non-pregnant controls. Whole blood was collected and the serum separated within 3 hr. 1 ml of serum was heated for 10 min in a water bath at 65°C. It was then transferred to the refrigerator for 10 min after which the serum was measured at 25°C according to the method of the DGKC using p-nitro- phenol phosphate as substrate and diethanolamine as buffer. The result was in the third trimester at >38 weeks, PALP isoenzyme becomes the most predominant isoenzyme in maternal circulation around 46% from TALP compared to non- pregnant mothers .This suggested that PALP isoenzyme are responsible for the significant increases of TALP during pregnancy advance (18).

A study conducted in Nigeria to monitor the changes in the level of HSAP in the serum during pregnancy and to correlate results with placental and birth weights among 411 normal and 213 pregnant women with low mean corpuscular hemoglobin, (< 27 pg) were included as control group. Serum samples were collected at the 15-22, 23-30 and 31-37weeks for analysis. The result of the HSAP activity in the serum was 53% in the 31-37 weeks, 40% in 23-30 weeks and 7.6% in 15-22 gestational weeks. The low MCH groups were 4.6%, 22.7% and 36.8% respectively. Neonatal birth weight mean values (kg) for normal and low MCH pregnancy are: M = 3.4 ± 0.6, F = 3.0 ± 0.8 vs M = 2.7 ± 0.7, F = 2.6 ± 0.6, respectively. They indicated determination of HSAP in serum could be diagnostic tools for assessing foetoplacental development and maternal health (19).

Another study in Western Nigeria was done to examine the effect of ethnic variation on PLAP activity values and the consequent effect of fetal nutrition. 290 pregnant women in apparent good health and within 30-35 week gestation period were randomly selected from five ethnic groups (Bini, Ibo, Ijaw, Itsekiri, and Urhobo). Serum was heated at 65°C for 7 min, and after allowing cooling, PLAP isoenzyme activity was then assayed by the Thymolphthalein Monophosphate

method. Investigated that PLAP isoenzyme activity value was highest among the Binis (121+11 IU/L), and lowest among the Ijaws (115+10 IU/L; P<0.05). These differences were significant (P<0.05). PLAP isoenzyme activity appears to contribute to fetal nutrition but such contribution varies among the ethnic groups. Hence, ethnic variation in PLAP isoenzyme genotype may be associated with differences in the enzyme activity. Thus, determination of PLAP isoenzyme should be performed among various ethnic groups (20).

In south Indian conducted a case control study on the clinical utility of ALP and PLAP isoenzyme as a reliable, sensitive, specific and economical best biochemical marker for the diagnosis of hypertensive disorder of pregnancy. Study included 60 pregnant women with hypertension and 60 controls.5 ml of blood sample was collected in a plain vacutainer then centrifuged at 1200 rpm for 10 min and separated the serum. 0.5 ml of sera samples were heated at 65 0C for 30 minutes. The ALP activities of the processed samples and the normal sample were carried out on the same day within four hours by the IFCC approved procedure using fully automated random access chemistry analyzer. Serum total ALP, PLAP and PLAP/ALP ratio levels were significantly higher in hypertensive pregnant women when compared to controls (P<0.05). There was significant correlation among ALP, PLAP and DBP. ROC analysis of ALP (169.5), PLAP (69) and PLAP/ALP (0.44) ratios showed optimum cut-offs in diagnosis of hypertension in pregnancy. The heat-stable ALP isoenzyme would be used as a best biomarker for the assessment of hypertensive disorders of pregnancy (21).

In Italy investigated a study on the interaction between PLAP isoenzyme and ABO system polymorphisms during intrauterine life among 903 newborns from a white population and 264 from Negro populations. Placental extracts were prepared by n-butanol as described by Boyer; starch gel electrophoresis at PH 6.0 and 8.6 was performed according to Robson and Harris, and enzymatic activity was developed by the method of Boyer.ABO and Rh blood types were determined in all infants (on cord blood) and in their mothers, and a direct Coombs test was performed on cord blood. The results shows, there is a difference in the PLAP isoenzyme distribution among ABO phenotypes; a significant deficiency was seen in the B group firstborn compatible infants as compared with the incompatible ones. An interaction between PLAP and ABO system polymorphisms which may take place during intrauterine life in both the white and

the Negro populations and it may play a role in ovum implantation and has some function in the regulation of the maternal immunological response toward the fetus (22).

A cross-sectional study was conducted in Khartoum state to compared the levels of liver function tests in grand multiparas with nulliparas [control] and primiparas among 100 nulliparity, 100 primiparity, and 10 grand multiparity between 20^{th}- 30^{th} weeks gestation.7 ml of venous blood was obtained in serum separator tubes. After 15 minutes the sample was centrifuged at 3000 rpm for 5 minutes.The test was carried out by Hitachi 912 full automated Chemistry Analyzer (Roche Diagnostics, Germany) as manufacturer procedure. Results showed, ALP significant low results in multiparity when compared to primiparity and control group, also, significant increase in primiparity as well as multiparity when compared to control group. They suggested vomiting in primiparity leads ALP increase compare with the reduction of ALP level in multiparity and its importance begins decreases with increased parity (23).

In Iran study was done to assessed the relationship between the serum calcium, phosphorous and alkaline Phosphatase levels in different trimesters of pregnancy and the effect of parity and the mother`s age. The serum sample was analyzed by a single reference laboratory. The General Linear Model Test result shows, both gestational age and parity, simultaneously affected the serum ALP level. There was remarkable correlation between the mean serum ALP with different trimesters of pregnancy (P= 0.007).Also, the mean level of serum ALP in the multiparous group was significantly less than the nuliparous group (P= 0.000). ANOVAs test did not indicate to any exact correlation between dependent variables factors and the age of mother (P= 0.057) (24).

A study was done in Northern Ireland to estimate the serum level of total and heat-stable ALP in normal and abnormal pregnancies on a total of 212 study group, 71 normal pregnancies and 61 patients with preeclampsia from 26 - 41 weeks. Serum sample heating at 65^0C for 30 minutes and determined serially using a colorimetric method, results were shown the mean percentage of heat-stable to total ALP level during 3^{rd} trimester was 66.91% in normal pregnant mothers and in pregnant patients with threatened abortion also increased the level and this raising was more significant in those with a previous history of threatened abortion. In most patients who did not have a history of abortion during a previous pregnancy, this enzyme level did not increase significantly compared with patients who had had previous abortion. It is conclude that changes

in levels of TALP and PLAP isoenzyme may be indictor of the sign of placental disturbances (26).

Those findings were clearly shows the association between the status of pregnancy and pregnancies out come with serum total and placental alkaline phosphatase isoenzyme levels. Therefore, it would be necessary to evaluate serum TALP and PLAP isoenzyme at different stage in association with host and environmental factors during pregnancy and the results recommended using it as one of the routine clinical tests for pregnant mothers.

1.4. Significance of the study

This research work encourages and gives some knowledge on the potential use of to TALP and PLAP isoenzyme as a simple accessible and affordable biomarker for monitoring the status of pregnancy. Moreover, it can provide baseline information for lower and middle level health facilities without a need for advanced diagnostic facilities.

It will also provide baseline for further large scale studies and proper diagnosis and interpretation of diseases or complication association with pregnancy in our local setup.

2. Objective

2.1. General Objective

- To evaluate serum TALP and PLAP at second and third trimesters in normal pregnant mothers in our local setup.

2.2. Specific Objective

- To determine serum TALP with second and third trimesters
- To determine serum PLAP with second and third trimesters
- To determine the correlation of TALP with PLAP with second and third trimesters
- To examine the association of some host factors such as age of mothers, blood groups, blood pressure, parities and previous history of abortion with the serum TALP and PALP.

2.3. Hypothesis

- Serum TALP and PLAP appearance and the levels in maternal serum have strong association with second and third trimesters
- Serum TALP and PLAP levels have association with some host factors

3. Methodology

3.1. Study Design

A hospital based cross sectional study has been conducted among apparently healthy consenting pregnant mothers who are at second and third trimesters.

3.2. Study sites

The study has been conducted in Addis Ababa which is the capital city of Ethiopia at Zewditu Memorial Hospital (ZMH) which is located in kirkose sub city, Addis Ababa, Ethiopia. It is organized under Health Main Directorate, Addis Ababa city Administration Health Bureau. It provides medical service for civil people. ZMH has 18wards with 250 beds. There are 385 health care professionals with different levels and field of training. Based on the 2013/2014 annual report the hospital provides services for 112,472 outpatients and 9,152 inpatient 4,243deliveries as well as 2,481antenatal follow up.

3.3. Study period

The study has been conducted from July to Nov 2015.

3.4. Population

3.4.1. Source population

All apparently healthy pregnant mothers who attending antenatal follow up at Zewditu Memorial Hospital

3.4.2. Study population

All apparently healthy pregnant women who attending antenatal follow up at Zewditu Memorial Hospital within study period

3.4.3. Sampling procedures and sample size

The sampling method was based on consecutive sampling technique. Cross sectional studies were done to estimate a population parameter like prevalence of some disease in a community or finding the average value of some quantitative variable in a population. Since, the aim of this study was to evaluate serum TALP and PLAP in quantitatively in our local set up and also it was not well studied in Ethiopia. Therefore, in this study had used a single population proportion formula considering the following assumptions

N = number of study subject

P = 50% a rough estimate was used

d = margin of error of 0.05 with 95% confidence level

$Z_{1-\alpha/2}$ = 1.96 (level of significance)

$$N = Z_{1-\alpha/2}^2 P (1- P) / d^2$$

$$(1.96)^2 \times 0.5 (1- 0.5) / (0.05)^2 = 384$$

3.4.4. Inclusion and Exclusion criteria

3.4.4.1. Inclusion criteria

All apparently healthy pregnant mothers who attending antenatal follow up at Zewditu Memorial Hospital were taken as a study subject.

3.4.4.2. Exclusion criteria

Pregnant mothers with Hypertensive disorders, Diabetes mellitus, Drugs intake, Smoking habit, Liver, Renal, Bone diseases, ovarian cancer, or any other major illness

3.5. Study variables

3.5.1. Dependent Variable

Serum TALP and PLAP isoenzyme

3.5.2. Independent Variable

Gestational age, age of mother`s, blood group, blood pressure, number of parities, number of abortions.

Alanine transaminase, Gamma glutamyl transpeptidase and Creatinine had been used as exclusion criteria to assess liver, kidney status

3.6. Measurement and Data collection

3.6.1. Data Collection

A structured questionnaire was specifically design to obtained information which helps to select individuals according to the selection criteria of the study. The questions included socio demographic and clinical data like age of mothers, number of parities, blood group, blood pressure and gestational weeks based on the date of last menstrual period, clinical measurement of the fundal height, and ultrasonographic estimation and. Medical records had been used to confirm the information.

The data had been collected by the two senior nurses` and well trained for one day with the objective of the study and the data collection procedure. Both were at the antenatal clinic of the Zewditu memorial hospital

3.6.2. Blood Sample Collection

3 ml of whole blood had been collected from each volunteer pregnant mothers using disposable syringe in plain sterile without anticoagulant tube and leaved to clot for 30 - 45 minutes then serum separation was carried out by centrifugation at 4000 rpm for 5 minutes. The serum sample was kept maximum 3 hours at room temperature before transportation and transported by using triple packaging system at room temperature to Federal police referral hospital laboratory. Sample analysis was carried out for every specimen on the same day of collection.

3.6.3. Sample Analysis

The portion of sera sample was carried out TALP by using Human reagents 4-Nitrophenylphosphatas (4-NPP) substrate according to the recommendations of DGKC kinetic method. ALP hydrolyses 4 – Nitrophenyl phosphate (4 – NPP) at a pH of 10.3 and temperature of 37 ^{0}C to liberate 4 – Nitrophenol (yellow complex). The total serum ALP was obtained by increasing in absorbance value of the 4 – nitrophenol at 405 nm. Alanine transaminase by using AMS reagents the 2-oxoglutarate substrate according to SCE recommendations, Gamma

glutamyltranspeptidase by using Linear chemicals S.L. reagents carboxyl substrate based on IFCC recommendation and Creatinine by using Human regents based on Jaffe reaction method were analyzed to exclude the participant whether they were apparently health or not.

0.5 ml of sera with equal amount of physiological saline was heated at 65^0C for 30 min in a water bath to inactivate the other isoenzyme such as liver, bone, intestinal. It was then transferred to the refrigerator at -20 for 3 min before returning it to room temperature (1) and the PALP isoenzyme activity remaining in the serum was measured by the same method as described for the estimation of TALP.

All biochemical analysis was carried out using the automated Human Star 300 chemistry analyzer at Federal police referral hospital laboratory within the day of collection. After analysis the serum was stored between 4^0C - 8°C for 48 hour (retention time). Reference rang based on the method.

3.6.4. Data Management and Quality Control

3.6.4.1. Data Management

All questionnaires were checked for errors, completeness and logical consistency at the end of each day to ensure the quality of data by principal investigator. Each sample collection and processing were followed the standard operating procedure of the Zewditu memorial hospital laboratory department. Also, each laboratory analysis was followed the standard operating procedure of Federal police referral hospital clinical chemistry laboratory.

3.6.4.2. Quality Control

Sample analysis carried out by expert laboratory technologist in the Federal police referral hospital clinical chemistry laboratory department together with principal investigator. The analyzer had a properly recorded daily, weekly and monthly maintenance. The instrument between-day precision was measured using 20 day testing by Huma Trol normal and pathological quality control sera for each parameter to ensure the validity of the test. The laboratory results were cheeked with westerguard rules and the reference rang of the method by principal investigator. All those performances have been supervised by the quality assurance

supervisor of the laboratory. The laboratory participated in the external quality assurance programmers with excellent performance results.

3.7. Data Analysis and Interpretation

The data was analyzed using the Statistical Package for Social Sciences (SPSS) software, version 21. Distributions of the TALP and PLAP isoenzyme level were found to be skewed; hence non-parametric analysis using percentiles were used in measuring the variation. Spearmann Rank test for correlation. Chi- square was done for host factors based on existing literature demonstrating. $P<0.05$ was considered statistically significant.

3.8. Ethical Considerations

Before the start of the research processed ethical clearance has been secured from Research Ethical Committee of Addis Ababa University School of Medical Laboratory Sciences and the permission has also been obtained from the study hospital (Zewditu Memorial Hospital).The objectives of study had explained to all eligible subjects and informed written consent from each study subjects in their local language included for involvement and venepuncture.

3.9. Dissemination of results

This study on completion could serve as a reference material for antenatal clinic and obstetrics department, to researchers and policy makers to utilize PALP isoenzyme laboratory test as best biomarker for monitor the status of pregnancy. To reach these bodies the finalized paper will be submitted to School of Medical Laboratory Sciences, Addis Ababa University. So it could serve as a reference in the library. In addition, a copy of this material will be given to Addis Ababa Health Bureau and respective hospitals. The result will also be disseminated through publication in peer reviewed local and international journals and through presenting it in relevant conferences.

4. Result

4.1. Demographic and clinical characteristics

The response rate of this study was 87%, a total of 333 apparently health pregnant mother were participated. Table1.was showed the majority (75%) of the participant were within the age group of 21 – 30 years old. Most of them were Multiparous (58%). Also, 87.1% of them had systolic BP between 100-130 mmHg and while (88.6%) had diastolic BP less than 90 mmHg. The majority (70.6%) had no previous abortion.

Table1.Demographic, BP and Blood group characteristics of the study participants, Zewditu Memorial Hospital, Addis Ababa, January 2015

Characteristics		**Numbers (%) of N =333**
1, Mother`s age	a, 18-20	23 (6.9)
	b, 21-25	108(32.4)
	c,26-30	143 (42.9)
	d, 31-35	40 (12)
	e, 36-40	19 (5.7)
2, Gestational age	a, 2nd trimester	69(20.7)
	b, 3rd trimester	159 (47.7)
	c, Term	93 (29.9)
	d, 40+	12 (3.6)
3, Blood group	a, `A`	90 (27.1)
	b ,`B`	83 (24.9)
	c, `AB`	35 (10.5)
	d ,`O`	125 (37.5)
4, Systolic BP	a, 80 -90 mmHg	43 (12.9)
	b, 100 - 130 mmHg	290 (87.1)
5, Diastolic BP	a, 60-80 mmHg	295 (88.6)
	b, 81-90 mmHg	38 (11.4)
5, Parity	a, Primipare	116 (34.8)
	b, Multiparous	193 (58)
	c, Grand Multipare	24 (7.2)
6, **Abortion**	a, 0	235 (70.6)
	b, 1	64(19.2)
	c, 2	18 (5.4)
	d, 3	16 (4.8)

4.2. Precision Assessment

Figure 3summarizes between day precision results as compared with Huma Trol normal and pathological quality control sera that the value provided by manufacturer for chemistry analyzer. As shown in the figure, precision values (%coefficient of variation) calculated from the standard deviation (27) and (242) multiplied by hundred is 11.1% for normal QC and (95) standard deviation and (1240) multiplied by hundred is8.2% for pathological which are below the cutoff that calculated from the manufacturer (<12.6%) and (<12.5%) respectively

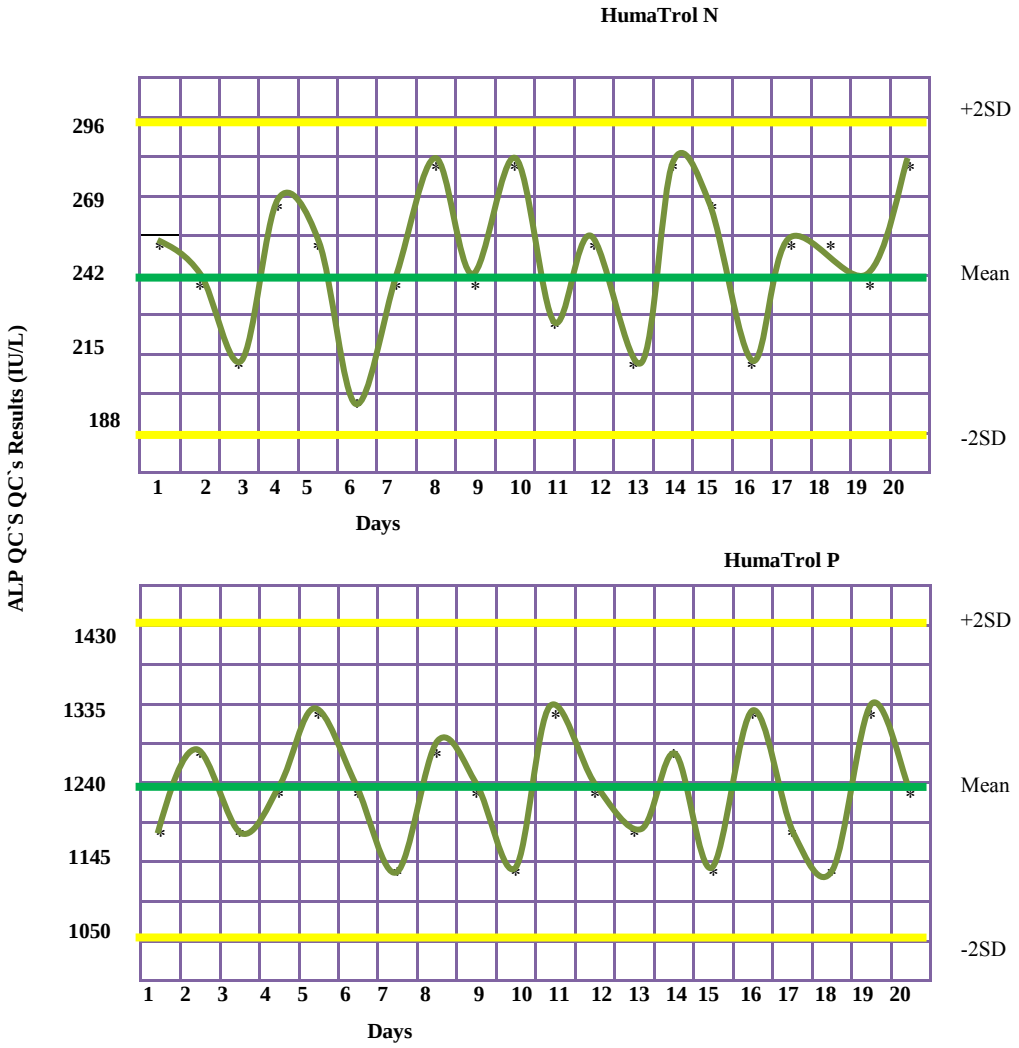

Figure3. Between days precision results to determined variation by using Hman Star 300 chemistry analyzer during study period, Federal police referral Hospital, Addis Ababa, January 2015

4.3. Distribution of central percentile serum TALP and PLAP isoenzyme levels in apparently health pregnant mothers

The 2.5 th, 50 th, 97.5 th percentiles serum TALP and PLAP isoenzyme levels were shown in Table 2. The central 95 percentile intervals of TALP and PLAP isoenzyme levels are found to be 121- 722 IU/L and 12-359 IU/L respectively.

Table2. The central 95 percentile interval TALP and PLAP isoenzyme levels in 333 apparently health pregnant mothers, Zewditu Memorial Hospital, Addis Ababa, January 2015

ALP level (IU/L)	**Median (2.5 th - 97 th) (IU/L)**
TALP	354 (121 – 722)
PLAP	154(12 – 359)
Overall (n = 333)	

The central 95 percentiles intervals of TALP and PLAP isoenzyme levels in relation to the gestational ages were shown in Table 3. TALP and PLAP isoenzyme levels in second trimester were (90–288 IU/L) and (4–62 IU/L) respectively, in third trimester were (137–617 IU/L) and (41-278 IU/L) respectively and at term (342-833 IU/L) and (163-401 IU/L) respectively, (P< 0001). There was a peak level of TALP and PLAP isoenzyme at term.

Table3.The central 95percentile intervals of TALP and PLAP isoenzyme levels in different gestational age groups in 333 apparently health pregnant mothers, Zewditu Memorial Hospital, Addis Ababa, January 2015

Gestational Age (N=333)	**Median (2.5 th - 97 th) (IU/L)**		PLAP from TALP
	TALP	PLAP	
Second Trimester (n = 69)	163 (90– 288)	25 (4 – 62)	15%
Third Trimester (n = 159)	334 (137 – 617)	141 (41 – 278)	41%
Term (n = 93)	449 (342– 833)	230 (163 – 401)	51%
40^{+} (n = 12)	385 (342-)	196 (154-)	
Overall (n = 333)			

4.4. Correlation assessment

The degree of correlation between TALP with PLAP isoenzyme was assessed by using Spearmann Rank test (Figure 4). Thus, statistically significant correlation observe between TALP with PLAP isoenzyme (r^2 = 0.968, p<0.001). Moreover gestational age of the pregnant mother were found to be significantly associated with TALP (r^2 = 0.805, p < 0.001) and PLAP isoenzyme. (r^2= 0.861, p<0.001).

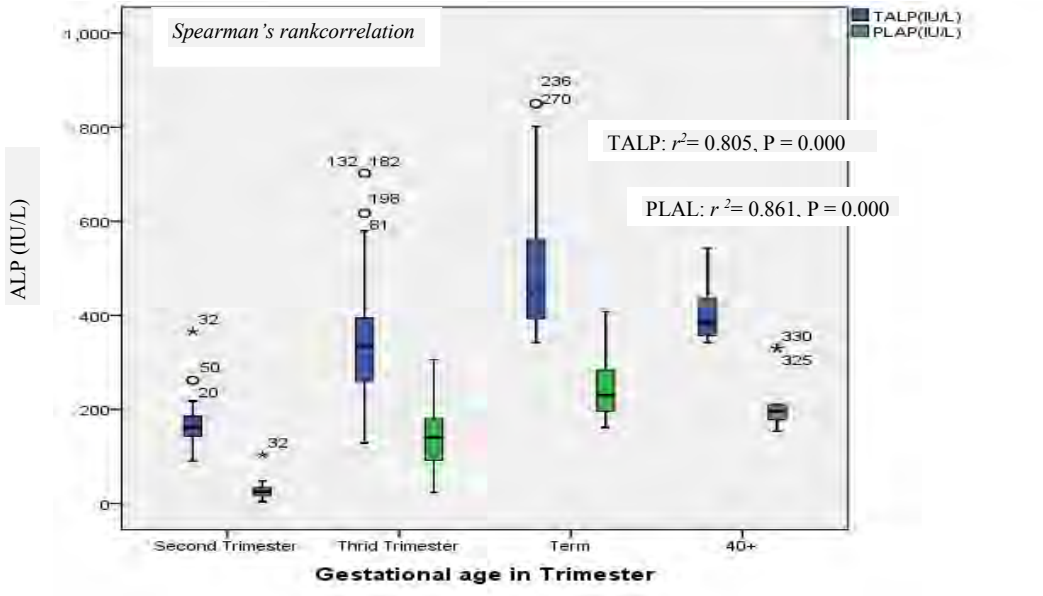

Figure4. Spearman`s rank correlation assessment of serum TALP and PLAP isoenzyme levels in different gestational age groups in 333 apparently health pregnant mothers, Zewditu Memorial Hospital, Addis Ababa, January 2015

4.5. Distributions of serum TALP and PLAP isoenzyme levels in some others maternal factors

Association and/or correlation results were shown in Table4 that the mean serum TALP and PLAP isoenzyme levels were associated in some host factors; there were significant positive association in both blood groups and blood pressure (P<0.05) where as others host factors were not statistically significant in this study(P>0.05).

Table4.Association of others host factors withserum TALP and PLAP levels in 333 apparently health pregnant mothers, Zewditu Memorial Hospital, Addis Ababa, January 2015

Characteristics	TALP		PLAP	
	X^2(d.f)	P-value	X^2 (d.f)	P-value
Mother`s age	8.596 (4)	0.072	5.802 (4)	0.214
Blood group	8.941 (3)	0.030	9.488 (3)	0.023
Systolic BP	12.430 (1)	0.001	12.756 (1)	0.001
Diastolic BP	3.885 (1)	0.044	4.693(1)	0.030
Parity	6.000 (2)	0.050	5.992 (2)	0.050
Previous history of abortion	1.26 5 (3)	0.737	1.631 (3)	0.652

Kruskal-Wallis rank test

4.5.1. Distribution of serum TALP and PLAP isoenzyme levels in different age groups

There was no statistically significant difference in serum TALP and PLAP levels in different age groups of apparently health pregnant mothers in this study, but there was highest levels in age group (18-20) and slight increase in age groups (36-40) (Figure5).

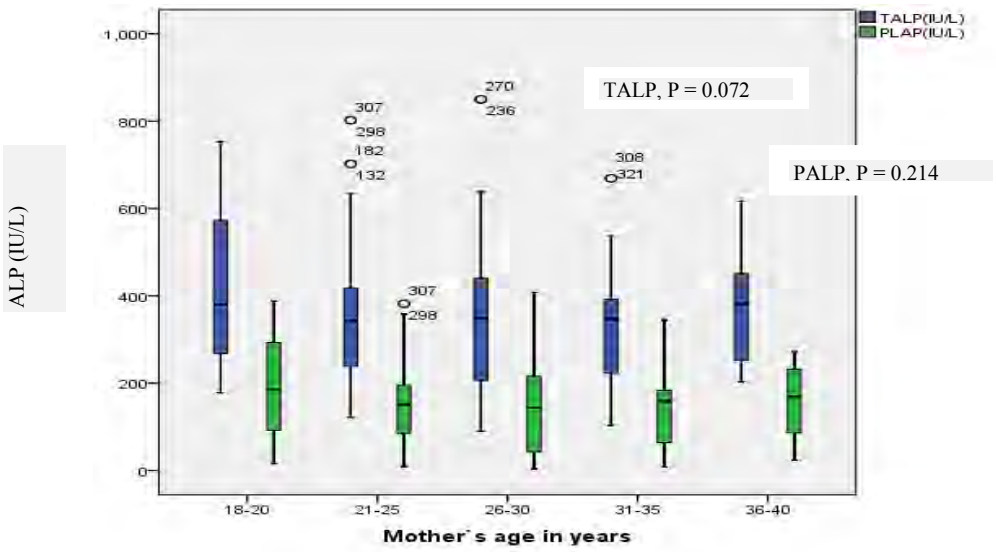

Figure5.Distribution of serum TALP and PLAP isoenzyme levels in different age groups of 333 apparently health pregnant mothers, Zewditu Memorial Hospital, Addis Ababa, January 2015

4.5.2. Distribution of serum TALP and PLAP isoenzyme levels in different blood groups

Figure6 has shown the different distribution of the levels of maternal serum TALP and PLAP isoenzyme in different blood groups of apparently health pregnant mothers; the `B` blood group had the lowest levels and ``A`` had the highest value. There was significant positive association between serum TALP and PLAP levels with ABO blood groups (P<0.05).

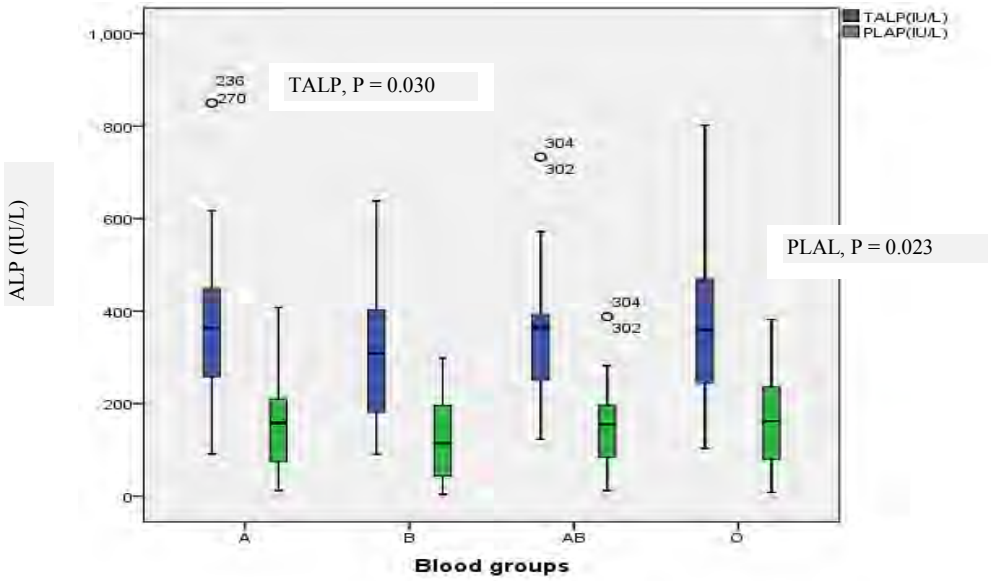

Figure6.Distribution of serum TALP and PLAP isoenzyme levels in different blood groups of 333 apparently health pregnant mothers in this study, Zewditu Memorial Hospital, Addis Ababa, January 2015

4.5.3. Distribution of serum TALP and PLAP isoenzyme levels in different blood pressure levels

We have seen in figure7 there was different distribution in the levels of maternal serum TALP and PLAP isoenzyme in different blood pressure levels. The value increased with increased blood pressure. It showed significant positive association between TALP and PLAP with both systolic and diastolic BP (P<0.05).

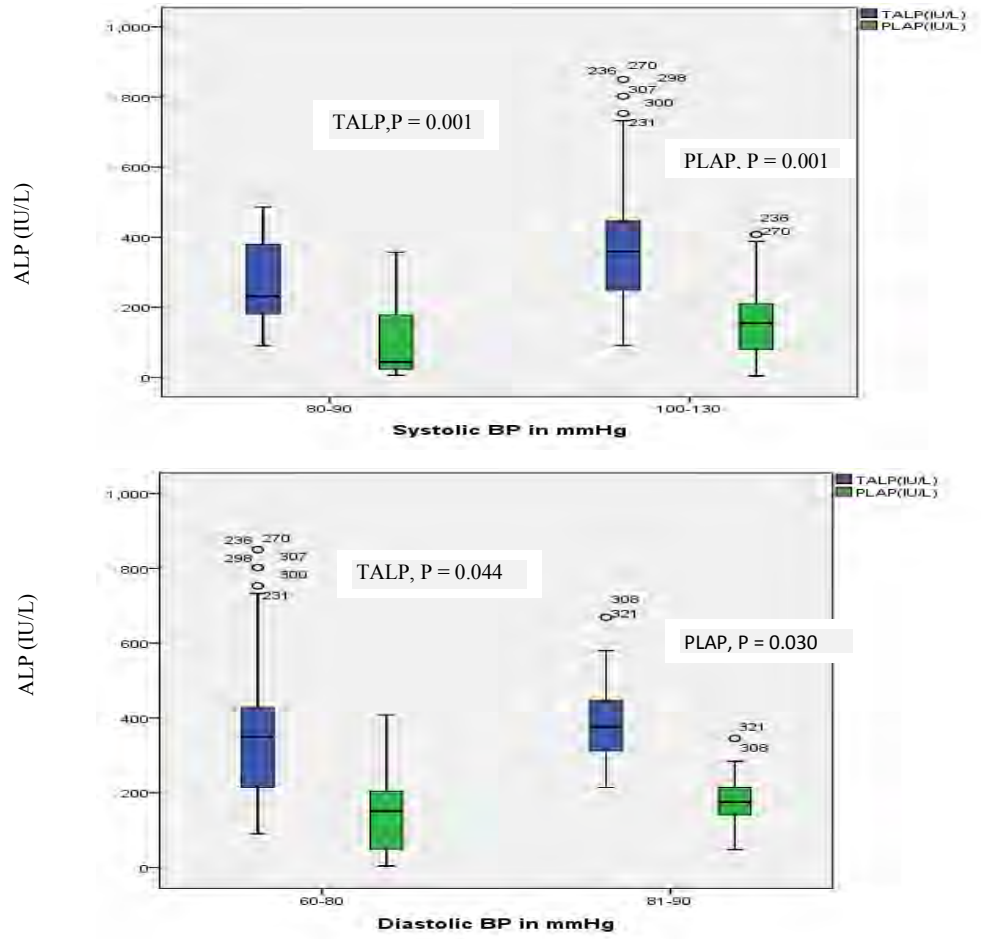

Figure7. Distribution of serum TALP and PLAP isoenzyme levels in different blood pressure levelsin333 apparently health pregnant mothers, Zewditu Memorial Hospital, Addis Ababa, January 2015

4.5.4. Distribution of serum TALP and PLAP isoenzyme levels in different parities and number of previous history of abortion

The distribution of serum TALP and PLAP isoenzyme levels showed in Figure 8 and 9decreased slightly with increased parities especially in grand multipara; multipara had high levels than grand multipara but it was not statistically significant (p= 0.05).Also, there was no statistically significant difference between the pregnant mothers who had no previous history of abortion with the mothers who had previous history of abortion. PLAP but it was not statistically significant (p> 0.05). In this study parities shows a slightly inverse association.

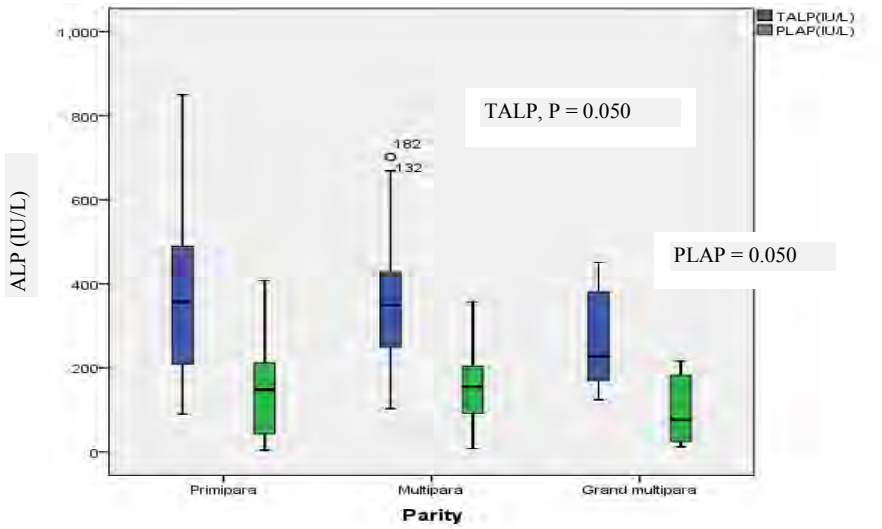

Figure8. Distribution of serum TALP and PLAP isoenzyme levels in different parities in 333 apparently health pregnant mothers, Zewditu Memorial Hospital, Addis Ababa, January 2015

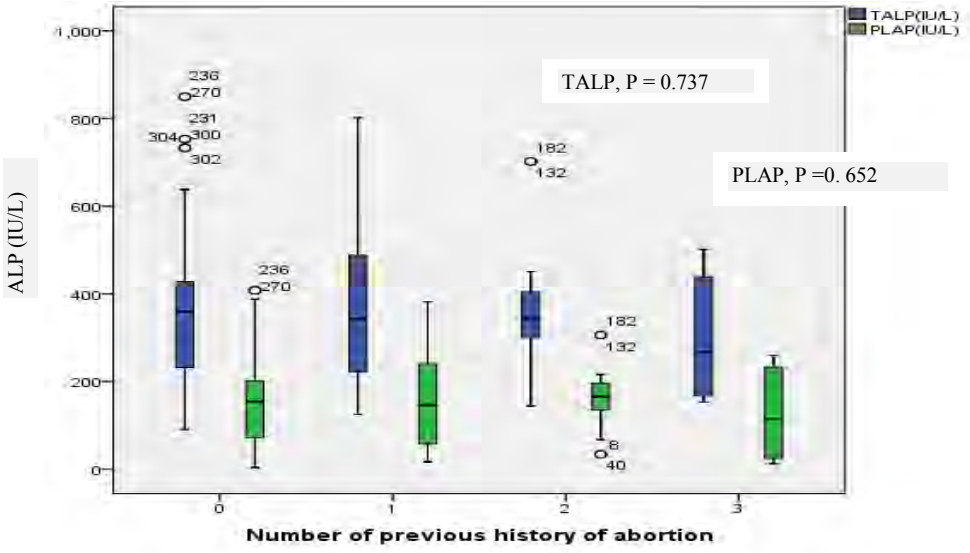

Figure9.Distribution of serum TALP and PLAP isoenzyme levels in number of previous history of abortion in 333 apparently health pregnant mothers, Zewditu Memorial Hospital, Addis Ababa, January 2015

5. Discussion

In this cross sectional study, a total of 333 randomly selected pregnant mothers included. From them majority were in third trimester. The maternal serum TALP and PLAP isoenzyme levels increased gradually throughout gestational age. There was a peak level of TALP and PLAP isoenzyme at term. A sudden drop was observed at the gestational age of 40 weeks and above, which was similar to those seen in other studies that the serum level of TALP increases 2-to 3-fold the upper limit of the reference range for non-pregnant women due to the appears of PALP isoenzyme. It becomes a major ALP in the maternal blood circulation towards term. These results were similar with previous finding (16, 17).

The central 95 percentiles of TALP and PLAP isoenzyme levels in second trimester were (90–288 IU/L) and (4–62 IU/L) respectively, in third trimester were (137–617 IU/L) and (41-278 IU/L) respectively and at term (342-833 IU/L) and (163-401 IU/L) respectively, (P< 0001). This data consistent with the data of previous finding that mean of serum TALP activity was(195.8 ± 34.7) in 2rdtrimester and (399.1 ± 147.4) 3rd trimester (2).Also, in line with the previous studies TALP and PLAP between 13-16 weeks were (59-121IU/L) and (0-8IU/L) respectively, 31-32 weeks were (91-219IU/L) and (19-97IU/L) respectively and >38 weeks (149-366IU/L) and (73-167IU/L) respectively; PALP isoenzyme around 46% from TALP compared to non- pregnant mothers (18).

The percent proportion of PLAP activity in this study was 51% in term, 41% in third trimester and 15% second trimester of TALP in the maternal serum .these in line with the previous studies 53% in the 31-37 weeks, 40% in 23-30 weeks and 7.6% in 15-22 gestational weeks(19).There was a strong positive correlation between TALP with PALP isoenzyme (r^2 = 0.968, p<0.001), TALP with gestational age (r^2 = 0.801, p < 0.001) and PALP isoenzyme with gestational age (r^2 = 0.861, p<0.001).These correlates well with the previous studies that PLAP Vs TALP 0.793 (P<0.05) (21).

This study has shown positive relationships between blood pressure and the levels of serum TALP and PLAP. In both the systolic and diastolic blood pressures of the study participant correlated positively with the serum levels of TALP and PLAP (P <0.05); the higher the systolic

and diastolic blood pressure, the higher the serum levels of TALP and PLAP. This result was similar with findings of others study that the mean values of ALP in normal pregnant women between 36-40 weeks was 206.13 ± 39 IU/L and the PLAP levels were 93.8 ± 17.4 IU/L. which reflects that the PLAP contributes 45% of the TALP in pregnant women with normal BP. Therefore, it may be used as early diagnosis and management of high blood pressure disorder (12, 21).

In present study has examined the different distribution of the levels of serum TALP and PALP isoenzyme among ABO blood groups, mothers whose blood group was in the B group had significant lower PALP isoenzyme value than others ($p<0.05$), This finding line with previous that there was a difference in PLAP distribution among ABO phenotypes; significant deficiency was seen in the B group newborn compatible infants with their mothers as compared with the incompatible ones. They suggested the occurrence of jaundice in newborn infants incompatible with their mothers in the ABO system appears to be associated with PLAP phenotypes (22).

The serum levels of TALP and PALP in Grand Multipare group less than multiparous group less than the Primipare group but not statistically significance ($P=0.050$), this might be because of unequal distribution of number of parity, the result indicates a negative association with number of parities. This results were seen in previous findings that the results were statistically significant ($P<0.001$), this inverse relationship have been proposed that vomiting in primiparity leads ALP increase compare with the reduction of ALP levels in multiparity and its importance begins decreases with increased parity(23,24).

In present study, there was no significant difference in the levels of serum TALP and PALP isoenzyme ($p> 0.05$) among mother`s age groups, but we have seen the highest in age groups between (18-20) and (36-40). This research result confirm with previous results that not indicated to any exact correlation between TALP and the age of mother ($P> 0.05$). The levels of serum TALP and PALP isoenzyme were no associated with the maternal age groups (24).

There was no difference between the mothers who did not have previous history of abortion with mothers who had in this study. This finding line with previous that in 14 pregnant who had two previous history of abortion the serum HSAP levels were in the normal rang, though in 4 cases

the levels were abnormal at some stage. They showed that the levels in case of bad obstetric history corresponded well to the condition of placenta, being normal in case with normal placental function and abnormal in others with failing placental function (25). In other hand, the HSAP level during 3rd trimester was 66.91% in normal pregnant mothers and this raising was more significant in those with a previous history of threatened abortion. They concluded that changes in levels of TALP and PLAP isoenzyme may be indictor of the sign of placental disturbances (26)

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6. Strengths and Limitation

6.1. Strengths

This study was attempts to control for confounding factors by taking into consideration others host factors that could possibly associated with the levels of TALP and PLAP

Even so, there are studies on the levels of serum TALP and PLAP elsewhere; this is the first study in Ethiopia up to our knowledge.

6.2. Limitation

Sample size may have been a limitation to this study.

7. Conclusion and Recommendation

7.1. Conclusion

- This study has shown that there was significant increasing serum TALP and PLAP isoenzyme levels with advancing gestational age. There was a peak level of TALP and PLAP isoenzyme at term. A sudden drop was observed at the gestational age of 40 weeks and above.
- A strong positive correlation observed between TALP and PLAP isoenzyme levels with second trimester, third trimester and term.
- Also, some host factors such as blood pressure and blood groups have shown positive relationships with the levels of serum TALP and PLAP. But the age of mother`s, number of parities and number of previous history of abortion were not significantly associated but have seen lower the value in grand multipara than in multipara.

7.2. Recommendation

- PLAP isoenzyme is an easy accessible and affordable test to monitoring the status of pregnancy at second and third trimesters especially in lower and middle level health facilities without a need for advanced diagnostic facilities
- The findings of this study centered on apparently healthy pregnant mothers, it may be interesting to use as baseline for another study
- According to this and previous research findings TALP and PLAP isoenzyme levels an early indicator of the status of pregnancy and pregnancy related complication such as high blood pressure, preterm delivery and others complication.

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Annexes

Annex I: English version of participant information sheet, consent and Information for the participant.

I. Participant information sheet

Addis Ababa University, School of Allied Health Science, Department of Medical Laboratory Sciences

Title of the Research: Serum Total and Placental Alkaline Phosphatase at Second and Third Trimester among Normal Pregnant Mothers at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

First I would like to thank you in advance for your cooperation and consent in Participation in this study. Please read or listen when it is read for you about the general information of the study. Your name will not be written in the form and assure you all the information you give will be kept strictly confidential. You are not obliged to answer any questions that you do not want to answer. If you are not comfortable with the questions please feel free to stop. With your permission, I will use a medical recorder to ensure accuracy of the data collection.

Background information

Alkaline phosphatase (ALP) (EC 3.1.3.1.) is an enzyme which catalyzes the hydrolysis of phosphate esters at an alkaline PH. Alkaline phosphatase is present in many tissues of the body with especially high level in the liver, bone, placenta and intestine. PLAP has several vital role during pregnancy for supporting fatal growth and development such as DNA synthesis and cell proliferation of the fetal tissue, transport nutrients and IGg to the developing fetus for defends.

Aim of the study

The purpose of this study is to evaluate Serum Total and Placental Alkaline Phosphatase at Second and Third Trimesters among Normal Pregnant Mothers.

Benefits for the participants

Study participants will not have any financial incentives or other inducements from participating on this study. However, based on the result you will have a series follow up. Most importantly, the result of the study will be beneficial to design for the utilization of placental ALP as simple, reliable and economically best biochemical markers for monitoring of the status of pregnancy especially in small and medium sized diagnostic laboratories. Hence, you are indirectly benefiting other pregnant mothers and the society in this respect.

Risks and complication

There are no anticipated risks to your participation. Venus blood sample will be collected once. During blood collection you may feel some discomfort but this does not produce serious pain.

Confidentiality

There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. Participants will not be prohibited from the study. Only interested participants can retrieve their own laboratory result using their code number. The information collected about you will be coded using numbers. No personal information will be disclosed to third party or will not appear in any report from this study.

Assurance of Principal Investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for advisor.

Serkalem Hailu (PI) Signature:_____ Date:_____

PI Address: Serkalem Hailu: Addis Ababa University, College of Health Sciences, School of Allied Health Science Department of Medical Laboratory Sciences
E-mail: serhail3@gmail.comTel:+25191178 91 57

II. Informed consent

I have been informed about the objective of the study entitled "Serum Total and Placental Alkaline Phosphatas isoenzyme at Second and Third Trimesters among Normal Pregnant Mothers at Zewditu Memorial Hospital, Addis Ababa, Ethiopia. I am also informed that all information contained within the questionnaire is to be kept confidential. In addition, I have been well informed of my right to reject information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care.

Therefore, with full understanding of the situation I agree to give the entire necessary information and blood sample for laboratory analysis. I have had the opportunity to ask questions about the study and received clarification to my satisfaction. I was also told that the results of the blood analysis will be given to the responsible health facility.

I _____ hereby give my consent for giving of the requested information and specimen for this study.

Participant cod: _____ Signature: _____

Date: _____

III. Information for the participant

Dear participants of this study, I appreciate in advance for being part of this study and providing genuine information.

Section 1- Socio-Demographic Characteristics

(1)Respondent's Identification code | | | | | | D | M | Yr |

(2) Address; Region_____ Town_____ Urban ☐ Rural ☐

(3) Age_____ (yrs)

Section 2- Clinical Characteristics

(4) Blood Group ----------

(5) Blood pressure; Systolic _____ Diastolic _____

(6) Gestational age---------- (in weeks).

(7) Previous obstetric history Parity? Yes ☐ No ☐, Number of delivery_____

(8) Previous obstetric history; Abortion? Yes ☐ No☐, Number of abortion _____

Dear participant, Thank you very much for taking your time and due concern.

Annex II: Procedure for Blood Collection, Processing and Analysis

I. Laboratory procedure for blood collection and processing

Based on Burtis CA, Aschwood ER and Bruns DE. Tietz Text book of Clinical chemistry and molecular diagnostic, 5th ed.*Elsevier Inc.,* 2012

1. Preparing blood collection materials.
2. Explain the blood drawing procedure to the client and reassure her.
3. Label tubes with the client's name/identification number.
4. Wear the rubber gloves and make the patient a comfortable position
5. Tie the tourniquet around the arm of the patient just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.
6. Using the tip of the index finger examine the phlebotomy site , feel the vein, and decide exactly where to place the puncture
7. Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab.
8. Insert the needle directly into the vein and withdraw peripheral blood of approximately 3ml in vacutainer tube
9. Withdraw the needle from the vein and cover the puncture site cotton swab and hold pressure at the puncture site for 3 minutes.
10. Properly discard the used materials in a safe container.
11. Leave for 30 to 45 min. to clot the blood
12. Centrifuge at 4000 rpm for 5 minutes and Serum will separate within half an hour and divide in to two portions.
13. One portion of the sera sample for the estimation of TALP, Alanine transaminase, Gamma glutamyltranspeptidase and Creatinin. The second portion for measuring PLAP

II. Determination of Total Alkaline phosphatase

TALP had been determined using standard DGKC Kinetic method

Principle

The enzyme alkaline phosphatase hydrolizes the p-nitrophenylphosphate (4-NPP) to releasing the p-nitrophenol (4-NPP) whose formation rate can be measured spectrophotometrically a 405nm to quantify the activity of the enzyme present in the sample.

Sample

Serum sample, Do not use hemolyzed samples. Sera kept at room temperature usually show a slight increase in activity, which varies from 1 % over a 6-h period to 3-6% over a 1 to 4 day's period. Even in sera stored at refrigerator temperature, activity increases slowly. In frozen sera, activity decreases but slowly recovers after thawing the serum.

Reagent preparation and stability

Bring the reagents to room temperature (15-25°C) before use, prepare working solution according to the kit`s instructions and wait 5minutes before use. The working solution is stable3days at 15-25°C or 3 weeks at 2-8°C when stored tightly closed, away from light.

Procedure

1. Preparing testing materials.
2. Perform at least two levels of an appropriate quality control material as sample and verify that the values obtained are within the reference range.
3. Pipette 300 µl of working solution and 6µl of sample.
4. Mix, incubate at 37°C for 1 minute, read the initial absorbance against water at 405nmin spectrophotometry. Make 3 readings at a distance of 60 seconds.
5. Calculate the average value of the absorbance variations per minute (ΔA/min)
6. Perform calculation in Units per liter, multiplying the ΔA/min by the factor as

Factor = 1000 x Tv/e x b x Sv

=1000x306µl /18.75x1x 6µl = 306µl /0.1125

F = 2720µl

ALP (U/L) = ΔA/min x 2720µl

Expected Value Female: 64 – 306 U/L

III. Determination of ALP Isoenzyme (Placental Alkaline Phosphatase)

PLAP isoenzyme had been determined using by Heat Inactivation Method

Based on Burtis CA, Aschwood ER and Bruns DE.TietzText book of Clinical chemistry and molecular diagnostic, 5thed.*Elsevier Inc,* 2012

Implementation of heat inactivation method requires a very precise control of experimental conditions such as temperature and duration of incubation.

Procedure

1. 0.5 ml of sera sample with equal amount of physiological saline will add into small thin glass tubes place in thermostatically control water bath stabilize at 65^{0}Cto inactivate the other isoenzyme such as liver, bone, intestinal. The water level will be at least 3 cm above the samples.
2. Exactly following 30 minutes, the serum tubes will rapidly remove and place in an ice bath for 3 min before returning it to room temperature.
3. The ALP activities of the processed samples will be determine similar to that of Total ALP using standard DGKC Kinetic method and this represent the placental isoenzyme.

IV. Alanine transaminase (SCE) Kinetic Method

Optimized UV test according to the recommendations of Scandinavian Committee on Enzymes (SCE)

Principle

In presence of 2- oxoglutarate, L- alanine is transformed into pyruvate and glutamate by ALT in the sample. In presence of NADH and lactate dehydrogenase, pyruvate is converted into lactate and NAD. NADH oxidation in time unit, the rate of decrease NADH is directly proportional to ALT concentration in the sample at 340 nm.

Sample

Serum sample, Do not use hemolyzed samples. Loss of activity within 3 days : at 2-8% °C< 10% and 15-25% °C < 17%

Stability at -20% °C at least 3 months

Reagent preparation and stability

Bring the reagents to room temperature (15-25°C) before use, prepare working solution according to the kit`s instructions and wait 5minutes before use. The working solution is stable 5 days at 15-25°C and 28 days at 2-8°C.

Procedure

1. Preparing testing materials.
2. Perform at least two levels of an appropriate quality control material as sample and verify that the values obtained are within the reference range.
3. Pipette 1000 µl of working solution and 100µl of sample.
4. Mix, incubate at 37°C for 1 minute, read the initial absorbance against air at 340nm in spectrophotometry. Make 3 readings at a distance of 60seconds.
5. Calculate the average value of the absorbance variations per minute (ΔA/min)
6. Perform calculation in Units per liter, multiplying the ΔA/min by the factor as

ALP (U/L) = ΔA/min x 1746

Expected Value Women: 8 – 40 U/L

V. Gamma glutamyltranspeptidase (IFCC) Kinetic Method

Quantitative determination of glutamyltransferase (GGT) in serum

Principle

The GGT, in the presence of glycyl-glycine, splits the L-γ-glutamyl-3-carboxy-4-nitroanilide (carboxi-glupa) in L-γ-glutamyl-glycyl-glycine and 5-amino-2-nitrobenzoate. The absorbance change in time unit measured at 405nm is proportional to the enzyme activity in the sample.

Sample

Serum, Do not use hemolyzed samples. The GGT in serum is stable one week at 2-25°C.Store at -20°C for prolonged periods.

Reagent preparation and stability

Bring the reagents to room temperature (15-25°C) before use, prepare working solution according to the kit`s instructions and wait 5minutes before use. The working solution is stable 5 days at 15-25°C and 4 weeks at 2-8°C.

Procedure

1. Preparing testing materials.
2. Perform at least two levels of an appropriate quality control material as sample and verify that the values obtained are within the reference range.
3. Pipette 1000 µl of Working solution and 100µl of sample.
4. Mix, incubate at 37°C for 1 minute, read the initial absorbance against water at 405nm in spectrophotometry. Make 3 readings at a distance of 60seconds.
5. Calculate the average value of the absorbance variations per minute (ΔA/min)
6. Perform calculation in Units per liter, multiplying the ΔA/min by the factor as

ALP (U/L) = ΔA/min x 1158

Expected Value Women :< 35U/L

VI. Creatinin Jaffe`s Reaction Method

Quantitative determination of creatinine by Jaffe`s reaction

Principle

Creatinine and non-creatininechromagen in the sample react with picric acid in alkaline media to form an orange-red colored complex solution. During the first step (approximately 30sec), mostly the rapid non-creatininechromagen react whereas during the following phase (approximate 2min) mainly true creatinine reacts, and finally the slow non-creatininechromagen. The change in absorption during the second step is measured at 510nm. The absorbance of this complex is proportional to the creatinine concentration in the sample.

Creatinine + Picric acid $\xrightarrow{NaOH}$ Creatinine-picrate comple

Sample

Serum sample, Do not use hemolyzed samples. Creatinine appears stable for at 2-8°C or three months frozen.

Reagent preparation and stability

Reagents store at room temperature 15-30°C, prepare working solution according to the kit`s instructions. The working solution is stable for 8 hours at room temperature.

Procedure

1. Preparing testing materials.
7. Perform at least two levels of an appropriate quality control material as sample and verify that the values obtained are within the reference range.
2. Dispense 1000 µl of working solution into tubes labeled reagent blank, calibrator, control and sample then dispense 100µl of calibrator, control and sample respectively.
3. Mix, incubate at 37°C for 10minute, read the initial absorbance against reagent blank at 510nm in spectrophotometry. Make 2readings at a distance of 60seconds. The test sample should be read within 30 min after color development.
4. Perform calculation in gm per dl.

$$\text{Unknown (mg/dl)} = \frac{\text{Unknown Absorbance} \times \text{Calibrator concentration (mg/dl)}}{\text{Calibrator Absorbance}}$$

Expected Value **Female** 0.5 up to 0.9 gm/d

Annex III: Result reporting format

I. Test result reporting format

Specimen collection date: ---------- Participant code:---------- **Specimen type:---------- Date reported:----------** **Comments:----------**			
Parameters	**Participant results**	**Reference range**	**Units of measurement**
TALP			
PLAP			
Alanine transaminase			
Gamma glutamyltranspeptidas			
Creatinin			

Table5. Test result reporting format

II. QC`s results reporting format

Parameter :---------- **QC`s analysis date: ----------** **Comments:----------**	**Normal QC sera results**	**Pathological QC sera results**	**Units of measurement**

Table6. QC`s results reporting format

Annex IV. Amharic Version of the participant information sheet, Consent and Information for the participant

I. በአዲስአበባዩንቨርስቲህክየተሳታፊዎችየመረጃቅጽ

አርስት፡-በእናቶችደምውስጥያለአልካላይንፎስፋቴዝንጥረነገርአይነትመጠንበተለያየየእርግዝናወቅትበጤናማእርጉዝእናቶችበዘ ውዲቱመታሰቢያሆስፒታልአዲስአበባኢትዮጵያ፡፡

አጠቃላይመረጃ፡-በጥናቱላይበመሳተፎከልብእያመሰገንንከመወሰንዎበፊትይህንቅጽበትክክልአንብቡወይምሲነበብልዎበትክክ ልምናፋክልቲየህክምናላቦራቶሪሳይንስትምህርትቤት

ያዳምጡ፤እንዲሁምግልጽያልሆነልዎትንነገርበሙሉበነጻነትይጠይቁ፡፡

አልካላይንፎስፋቴዝንጥረነገርአይነቶችበተለያዩየሰውነታችንክፍሎችይመነጫሉበተለይበጉበት፣በአጥንት፣በአንጀትእናበእንግዴ ልጅ፡፡ከእንግዴልጅሚመነጨውንጥረነገርለሽሉወይምለህጻኑበጣምትልቅአገልግሎትይሰጣልምግብበማመላለስእናበሽታንበመ ከላከል፡፡በእርግዝናወቅትከ2-3
እጥፍይጨምራልእርጉዝካልሆኑትሴቶችበተጨማሪምከእርግዝናጋርበተያያዘበሽታሊጨምርወይምሊቀንስይችላል፡፡ስለዚህይህ ንጥረነገርበየጊዜውመጠኑመታየትአለበት፡፡

የጥናቱአላማ፡-በእናቶችደምውስጥያለአልካላይንፎስፋቴዝንጥረነገርአይነትመጠንበተለያየየእርግዝናወቅትበጤናማእርጉዝእናቶ ችመገምገምነው፡፡

ጥናቱለተሳታፊዎትያለውጥቅም፡-በጥናቱለሚሳተፉፍቃደኛተሳታፊዎችምንምአይነትየገንዘብክፍያየለም፣ነገርግንበምርመራው ውጤትመሰረትበደንበየመታየትእናየመታከምእድልይኖሮታል፡፡በተጨማሪምየጥናቱውጤትበእርግዝናወቅትያለውንሁኔታለመ ገምገምስለሚጠቅምበተዘዋዋሪመንገድሌላእርጉዝሴቶችእናህብረተሰቡንየመጥቀምእድልያገኛሉ፡፡

በጥናቱተሳታፊውላይያለውጉዳትናተዛማጅችግር

በዚህጥናትበመሳተፍሊደርስብዎየሚችልምንምጉዳትአይኖርምለዚህጥናትምርመራየሚውልየደምናሙናየሚወሰድሲሆንከመጠ ነኛስሜትበስተቀርበጤናዎላይምንምጉዳትአይደርስቦትም፡፡

የመረጃሚስጥራዊአጠባበቅ፦የሚሰጡትመረጃበወቅቱምሆነከዛበኃላባሉትጊዜያትመሉበመሉሚስጥ ራዊነቱየሚጠበቅናመረጃውምበስምሳይሆንበመለያቁጥርይሆናል፡፡በጥናቱላይመሳተፍካልፈለጉየማቆ ምመብትአልዎት፡፡የላቦራቶሪውጤትዎንከፈለጉየመለያቁጥሮንበመጠቀምበሚሰጥትየቀጠሮጊዜመ ውሰድይችላሉ፡፡

ጥናቱንየሚያካሄደውሰውማረጋገጫ፦ለዚህጥናትሃላፊነቱንለመውሰድና፣ማናቸውንምጥናቱየሚመለ ከትጉዳዮችንክትትልለማድረግናለሚመለከተውአካልለማሳወቅበፊርማዬአረጋግጣለው፡፡

ፊርማ ---------- ቀን----------

ማንኛውንምጥያቄመጠየቅለሚፈልጉየሚቀጥለውንአድራሻዬንመጠቀምይችላሉ፡፡

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II. የፈቃደኝነትማረጋገጫቅጽ

በእናቶችደምውስጥያለአልካላይንፎስፋቴዝንጥረነገርአይነትመጠንበተለያየየእርግዝናወቅትበጤናማ እርጉዝእናቶችበሚልርዕስላይበተመለከተበሚደረገውጥናትላይየምሳተፍመሆኔ፤የጥናቱአላማናጥቅ ምተገልጾልኛል፡፡በመጠየቁላይየሚገለጸውየኔሙሉመረጃምበሚስጥርእንደሚያዝተገልጻል፡፡

በተጨማሪምበጥናቱላይአለመሳተፍመብቴእንደሆነበዚህምምክንያትምንምአይነትመጉላላትእንደማ ይደርስኝገብቶኛል፡፡ስለሆነምሁኔታውንበሚገባበማጤንበፍቃደኝነትበምርምሩላይለመሳተፍለተመራ ማሪውፍቃደኝነቴንሰጥቻለው፡፡የምሰጠውየደምናሙናለተጠቀሰውጥናትብቻእንደሚውልተገልጾልኝ ተስማምቻለሁ፡፡ማንኛውንምያልገባኝንነገርየመጠየቅእድልተሰጥቶኝመልስአግኝቻለሁ፡፡

በተጨማሪምየላቦራቶሪየምርመራውጤቶችንበጊዜውክትትልለሚያደርግልኝየጤናባለሙያእንደሚሰ ጡናማወቅምከፈለኩማግኘትእንደምችልተገልጾልኛል፡፡

እኔ----------
የተባልኩግለሰብይህንሁሉበመገንዘብበምርምሩላይስለእኔመረጃናየደምናሙናለመስጠትተስማምቻለ ሁ፡፡

የተሳታፊውመለያ ---------- ፊርማ---------- ቀን ----------

III. የተሳታፊውመግለጫ፦

ውድየዚህጥናትተሳታፊዎችየዚህጥናትአካልበመሆናችሁናትክክለኛሀሳባችሁንበመስጠታችሁከልብ እናመሰግናለን፡፡

ክፍል 1- የኑሮናየማህበራዊሁኔታ

(1) የተሳታፊውመለያ

ቀን	ወር	አመት

(2) አድራሻክልል_____ ከተማ_____ ገጠር _____

(3) እድሜ _____ (በአመት)

ክፍል 2- የህክምናመረጃ

(4) የደምአይነት----------

(5) የደምግፊት፦ሲስቶሊክ_____ዲያስቶሊክ _____

(6) የእርግዝናውጊዜ_____(በሳምንት)

(7) ከዚህበፊትየነበረየወሊድታሪክ፦የወሊድብዛት-------

(8) ከዚህበፊትየነበረየማሶረድታሪክ፦የማሶረድብዛት _____

ውድየዚህጥናትተሳታፊ፦ሀሳቦትእናጊዜዎትንበመስጠቶትከልብእናመሰግናለን፡፡

Annex V. Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Name: Serkalem Hailu (BSc) Signature _____

Place: Addis Ababa University College of Health Sciences, School of Allied Health Science Department of Medical Laboratory Sciences, Ethiopia

Date of submission: _____

This thesis has been submitted with my approval as University advisor.

Name: Samuel Kinde, BSc, MSc Signature _____

This thesis has been submitted with my approval as clinical advisor.

Name: Dr. Tilahun Kuma, Obstetrician/Gynecologist Signature _____