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Original Research Article

Biochemical analysis of Liver Function Test in different trimesters of Pregnancy

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Abstract

Background and objectives: Changes in value of certain serum liver function tests may be challenging and relies on laboratory investigations during pregnancy. So, it is important to distinguish the physiological changes or disease pathology. Therefore, this study aimed to estimate liver function test and its correlation with body mass index in pregnant women compared to non-pregnant healthy women in Terai of Nepal.
Material and Methods: The case control study was carried out in Department of Biochemistry at Clinical Pathology Laboratory, Janaki Medical College Teaching Hospital, Tribhuvan University, Nepal. Cluster random sampling was adopted. Blood samples were analyzed for liver function profile, and protein profile was processed with semi-automatic analyzer using clinical chemistry reagent kits. BMI was also calculated.
Results: The mean ALP level was slightly increased in 2nd trimester and drastically increased in 3rd trimesters during pregnancy. But, the mean ALT, protein and albumin level was decreased in the 2nd and 3rd trimester as compared to 1st as well as non-pregnant healthy women. BMI in pregnant women increased with the trimesters of pregnancy and was statistically significant. The minor variation in globulin in different trimesters was confirmed as compared to controls. BMI and ALP was positively correlated whereas albumin and total protein with BMI were negatively correlated. All the parameters were found to be highly significant. However, the relationship between ALT, AST and globulin with BMI was insignificant.
Conclusion: The ALP level was significantly elevated in 3rd trimester as compared to non-pregnant healthy women. Other liver enzyme, ALT was decreased and AST was found to be unaffected. A slight variation in albumin, globulin and total protein was depicted. Relative values of various liver function tests during gestational trimesters appear to be the best guide to confirm the diagnosis and treatment strategies. Thus, gynecologists should prefer LFTs as routine tests in all gestational trimesters to avoid the future complications to mother and offspring.
Keywords: Albumin, Body Mass Index, Liver function test, Pregnancy, Protein .

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1. Introduction

Women are considered as a supreme creature of God who undergoes through a variety of physiological changes to shore up the development and growth of the fetus during pregnancy. [1] Alteration in liver biochemical profile is normal during pregnancy. [2,3] The physiological changes in liver function are usually transient which remain only during pregnancy periods and, then it corrects spontaneously. But, identification of these physiological changes is vital for the diagnosis of liver disease. Although it is very rare during pregnancy but, can occur which must be predictable at preliminary phase to decrease morbidity and mortality for both mother and infant. [4] It is important to distinguish the physiological changes and disease pathology.

The pathological derangement in the liver functions may be related to pregnancy or may coexist with pregnancy and may be divided into three major groups [5].

First group includes liver disorders that are specific to pregnancy such as hyperemesis gravidarum, pre-eclampsia, eclampsia, syndrome of haemolysis, elevated liver test and low platelets (HELLP), acute fatty liver of pregnancy and intrahepatic cholestasis of pregnancy. These disorders are mostly trimester specific. Second group includes intercurrent liver disease occurring during pregnancy such as viral hepatitis and herpes simplex. Third group includes pregnancy with pre-existing liver disease such as chronic active hepatitis, Wilson's disease, cirrhosis of liver, portal hypertension, Budd-Chiary syndrome and hepatic tumour. [6-8]

Accurate biochemical analysis of liver function tests (LFTs) can lead to proper diagnosis and timely management of diseases and may diminish complications [3,9]. LFTs are commonly ordered panel of blood tests that assess liver function, liver damage and biliary system function including intra and extra hepatic bile ducts and gall bladder. [8,10] Additionally, LFTs evaluate aspects of physiology outside hepatobiliary system, for instance they provide insights into coagulation, haemolysis, nutrition, bone turnover and other conditions. [8,11,12] The specific tests included in LFTs are hepatic enzymes, synthetic function tests, and bilirubin. In common practice, there are four hepatic enzymes of importance: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT). [5,13]

AST and ALT are collectively known as aminotransferases or transaminases. AST is found in liver, cardiac muscles, brain, skeletal muscles, erythrocytes and kidney where as ALT is found predominantly in liver. Therefore, increases in ALT are more specific than AST for hepatobiliary disease. There is no agreement on the effect of pregnancy on serum AST and ALT. In a few studies, AST and ALT levels slightly increase in the third trimester. However, in most studies, AST and ALT levels remain within the normal range for non-pregnant state. Uterine muscle contractions during labor may increase AST or ALT activity levels. [14]

The enzyme ALP is a collection of isoenzymes present throughout the body. The most clinically relevant ALP is found in liver, bones, the biliary duct, white blood cells, small bowel and placenta. [11, 15] Serum ALP levels are elevated in late pregnancy, usually in the third trimester which is mostly due to the production of placental isoenzyme rather than elevation of hepatic isoenzymes while GGT activity is generally considered normal during gestation, emerging studies show decrease in serum GGT levels with gestational age. [8,14]

Albumin is a globular protein, formally called human serum albumin. It is produced by the liver and comprises about half of the total protein in blood. [16] Serum albumin concentration decreases with pregnancy age. The decrease in serum albumin levels is attributed to pregnancy-related plasma expansion. Indeed, intravascular albumin mass and the rate of metabolism in pregnant women is within the normal limits for non-pregnant women. [17, 18] Serum albumin levels fall leading to a relative rise in α-2-globulin whose renal loss is least. Theoretically, increased renal albumin loss should only cause a relative rise in tumor suppressor gene (TSG); however, there is the possibility that the liver, when attempting to replace excess albumin loss, overproduces serum globulin fractions. Changes in serum proteins during normal pregnancy are attributed to increase in plasma volume and dietary protein requirements. [19]

The diagnosis of liver disease in pregnancy is challenging and relies on laboratory investigations. Signs and symptoms are often not specific and consist of jaundice, nausea, vomiting, and abdominal pain. The physical examination of a pregnant woman can show skin changes suggesting chronic liver disease, such as palmar erythema and spider angiomas. These changes are the result of hyperesterogenemia of pregnancy and occur in up to 60% of healthy pregnancies. [8, 20]

Alterations of laboratory test results can represent physiological changes of pregnancy. Elevations of transaminase, bilirubin, and prothrombin time (PT) whereas decreased level of serum albumin and increased level of alkaline phosphatase indicate a pathological state. The unconjugated hyperbilirubinemia of Gilbert's syndrome is not affected by the pregnancy. Clotting factors are affected by normal pregnancy and favor a hypercoagulable state. Women with inherited thrombophilia, such as factor V Leiden or antithrombin III deficiency, are at increased risk for hepatic vein and portal vein thrombosis during pregnancy. [4,8]

Nepal has made substantial progress in improving maternal health care access and utilization. However, disparities remain according to women's socioeconomic status, education level and place of residence. In most of Terai at Province No. 2 in Nepal, women's health issues during pregnancy remain inappropriately addressed. Nepalese women of reproductive age suffer in different ways with several barriers during pregnancy. Pregnancies associated liver diseases affect up to 3% of pregnant women and are the most frequent cause of liver dysfunction in pregnancy. [14] Pregnancy is sometimes complicated by specific or non-specific liver diseases. [21]

It has been estimated that liver function test abnormalities occur in around 3%–5% of pregnancies. [22] Evidence across different obstetric populations is consistent that increased pre-pregnancy BMI associates with increased perinatal morbidity, including obstetrical interventions at birth such as labour induction and surgical deliveries. [23] The timing of clinical manifestations and liver function test result abnormalities can be critical for determining the di-

agnosis and treatment strategies. Therefore, it was judicious to study and evaluate liver function profile and plasma proteins along with its association with BMI in pregnant women compared to non-pregnant healthy women in Terai of Nepal.

2. Materials and methods

2.1 Study Setting

The present study was conducted at Department of Biochemistry in Clinical Pathology Laboratory from January 2015 to June 2017 in collaboration with Department of Obstetrics and Gynecology, Janaki Medical College Teaching Hospital, Ramdaiya, Bhawadi affiliated to Tribhuvan University, Nepal. It is situated in rural area Ramdiaya, Bhawadi of Dhanusha district in Province No. 2 of Nepal.

2.2 Study Subjects

A total of 336 study subjects were enrolled. 224 pregnant women (cases) and 112 non-pregnant healthy women (controls) were selected.

2.3 Inclusion Criteria

Cases: Pregnant women of reproductive age were confirmed by a validated pregnancy test report. Out of 224 cases (pregnant women), 126 cases were selected from JMCTH, Ramdaiya and 98 cases were from antenatal care clinics at sub-metropolitan city, Janakpurdham, Nepal.

Controls: A total of 112 controls (non-pregnant healthy women) were selected from Marie Stops Center, Janakpur at the same geographical region.

2.4 Exclusion criteria

Pregnant women with gestational diabetes mellitus, hypertension, obesity, and women with other chronic diseases, drug induced abnormal liver function test and women over age 40 were excluded.

2.5 Ethical consideration

The work approval was taken from the Research Ethical Review Board of Singhania University, Rajasthan, India. Additional ethical consent letter was also received from the Institutional Review Board of Janaki Medical College Teaching Hospital, Ramdaiya, Nepal. Written informed consent was obtained from the participants to ensure their voluntary participation before preceding the questionnaire and specimen collection.

2.6 Sampling Technique

Cluster random sampling was adopted for this case control study. A higher number of cases were decided without compromising the power of study based on available literature and studies. [24-26] Matching was done only for the age variable.

2.7 Questionnaire and Data collection

A structured questionnaire prepared for interview was designated for matching the study need. The questionnaire was based on Family Planning Association of Nepal and on other international studies with some modifications consulting Women Health Specialists. The questions were also focused on socio demographic data (age, sex, parity) and duration of pregnancy. After obtaining the demographic, menstrual and obstetric details, the specific symptoms related to liver dysfunction such as pruritus, persistent vomiting, yellowish discoloration of urine, blurring of vision, diminished urine output, upper abdominal discomfort and anorexia were asked.

2.8 Anthropometric measurement

All participants had their height and weight measured by trained healthcare professionals. The women were weighed with a digital scale (Filizola Ltd., São Paulo, Brazil), and their stature was measured with a Seca Portable Stadiometer (Seca Ltd., Hamburg, Germany). BMI was calculated by dividing weight in kilograms by the square of height in meters using the formula: Weight in kilograms/ (height in metres)^2. [27] Maternal pre-pregnancy BMI was categorized into underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obese (≥30.0 kg/m^2) groups on the basis of World Health Organization BMI classification. [28]

2.9 Blood Sample Collection

Under all aseptic conditions, venous blood samples were collected from the antecubital vein making the participants in the supine position prior for commencement of intravenous therapy. Blood samples were collected in a 3.5 ml vacutainer (BD Plymouth, PL6 7BP, UK, SST II Advance Tubes) and immediately transported to clinical pathology laboratory for the preparation of serum. Either the blood sample was immediately processed or stored in a freeze at 2-8°C. Then, the serum was separated by ultracentrifugation of the sample at room temperature at 3500 rpm for 10 min at 4°C and stored at −20 °C until analysis. Precautionary measures were taken to see that there was no hemolysis and if occured, hemolysed samples were discarded.

2.10 Clinical laboratory Techniques

Blood samples were analyzed for total protein, albumin, globulin, ALT, AST and ALP. Serum ALT and AST activity was measured by modified International federation of clinical chemistry (IFCC) kinetic method. Serum ALP activity was measured by p-Nitrophenyl Phosphate (pNPP) Kinetic method. Serum total protein concentration was estimated by Biuret end point method. Serum albumin concentration was measured by Bromocresol Green (BCG) end point method. Serum globulin concentration was calculated by subtracting albumin concentration from total protein concentration [3].

2.11 Instrumentation and Reference limits

All biochemical parameters were processed in semi automatic analyser (Humalyzer 3500) using commercially available clinical chemistry reagent kits provided by Human diagnostics worldwide, Weisbaden, Germany. Reference limits of normal values in laboratory

for non-pregnant women up to 34 U/L at 37º C for serum ALT, up to 31 U/L at 37º C for serum AST, 30 to 125 U/L at 37ºC for serum ALP, 6 to 8 gm/dl for serum total protein concentration, 3.5 to 5.3 gm/dl for serum albumin concentration, 2.3 to 3.6 gm/dl for serum globulin concentration.

2.12 Statistical Analysis

Data were entered and analyzed using SPSS version 20.0. Chi-square test (χ^2) and one way ANOVA was used to identify the significance of the relations, associations, and interactions among various variables. The p-value ($p<0.05$) was considered as statistical significant.

2.13 Quality control

Aseptic conditions and precautionary measures were taken prior to intravenous therapy. Manufacture, expiry date and proper storage for the reagents were regularly scrutinized. Temperature was monitored each day and corrected if any deviation observed. Hemolysed samples were discarded.

3. Results

3.1 Age distribution of participants

Age distribution of participants was as shown in table 1. The highest numbers of pregnant and non-pregnant healthy women were in between 20-30 years and lowest in between 30-40 years. Pattern of age between pregnant women and non-pregnant healthy women was found to be statistically significant (p = 0.4332).

Table 1: Age distribution of participants

Age (yrs)	Pregnant women (%)	Non-Pregnant Healthy women (%)	Total (%)	Statistics
Less than 20	58 (25.9)	26 (23.2)	84 (25)	χ^2= 1.67 p = 0.4332
20-30	154 (68.8)	76 (67.8)	224 (66.66)	
30-40	12 (5.4)	10 (8.9)	28 (8.36)	
Total	**224**	**112**	**336**	

3.2 Anthropometric measurement of study participants

The more number of study participants were in 2nd trimester of pregnancy followed by 3rd trimester as shown in figure 1. The mean weight of pregnant women in 1st, 2nd and 3rd trimester was 47.55, 53.69 and 56.73 with standard deviation of 4.46, 3.14, and 2.84 respectively and the figure in non-pregnant healthy women was 48.48 with SD of 4.08. The mean height in pregnant women was 153.66, 152.57 and 152.51 with standard deviation of 7.22, 6.67 and 7.58 in different trimesters respectively and in non-pregnant healthy women were 151.96 with SD of 7.01. The mean BMI in pregnant women was 20.24, 23.18 and 24.53 with standard deviation of 2.51, 2.36, and 2.31 in 1st, 2nd and 3rd trimester respectively and in non-pregnant healthy women was 21.10 with SD of 2.32. This depicts that weight and BMI in pregnant women increases with the trimesters of pregnancy and was statistically significant (p= 0.000).

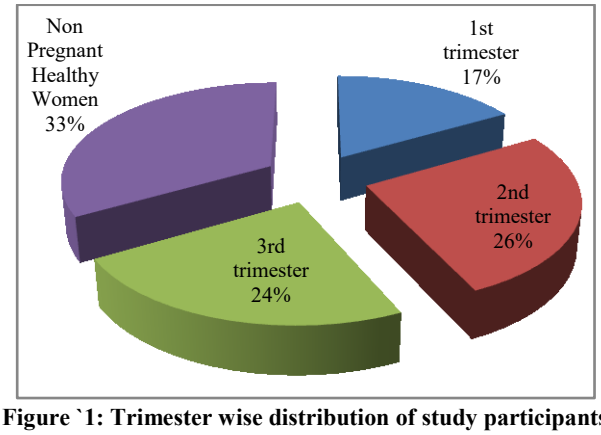

Figure `1: Trimester wise distribution of study participants

3.3 Participant's Obstetrics history

More than one third (38.39%) of women were of gravid one followed by 26.33%, 21.42% and 13.83% of gravid 2, 3 and 4 respectively. However, less than one third (29.01%) of women were nulliparous. Parity between 1 and 4 was prevalent in 70.99% as shown in table 2.

Table 2: Participant's Obstetrics history

Gravida	Number of participants (%)
1	86 (38.39)
2	59 (26.33)
3	48 (21.42)
4	31 (13.83)
Total	**224**
Parity	
0	65 (29.01)
1	52 (23.21)
2	50 (22.32)
3	38 (16.96)
4	19 (8.48)
Total	**224**

3.4 Liver fuction profile of subjects

Table 3 highlights the liver function profile (ALT, AST, ALP) (IU/L) of the study participants. The finding demonstrates the mean ALT level was decreased in 3rd and 2nd trimester as compared to 1st as well as non-pregnant healthy women (p=0.004). The F-value was 4.60 and the difference was significant. The small variation in AST activity was found in different trimesters of pregnancy as compared to controls. The F-value was 0.29 (p=0.830) statistically insignificant which shows AST level is unaffected during stage of pregnancy. The mean ALP level increased slightly in 2nd trimester and drastically increased in 3rd trimesters during pregnancy as compared to 1st trimester as well as non-pregnant healthy women. The difference was found to be statistically significant with F-value 164.98 (p=0.000).

Table 3: Liver function profile of participants

Liver function Profile (IU/L) at 37ºC	Pregnant Women (Trimesters) (n=224)			Non Pregnant Healthy Women (n=112)	F-value	p-value
	1st	2nd	3rd			
ALT	15.20±9.14	12.32±5.89	11.20±6.07	13.80±4.60	4.60	0.004
AST	18.50±7.99	18.52±6.00	18.53±6.59	19.22±6.43	0.29	0.830
ALP	72.32±21.38	79.57±15.37	126.97±15.97	105.58±15.88	164.98	0.000

(ALT-Alanine aminotransferse; AST-Aspartate amino transferase; ALP-Alkaline phosphatase)

3.5 Protein Profile of participants

Table 4 describes the protein profile (albumin, globulin, total protein) (gm/dl) of study subjects. The findings demonstrate the mean albumin level was decreased in the 3rd and 2nd trimester as compared to 1st as well as non-pregnant healthy women (p=0.000). The minor variation in globulin in different trimesters as compared to controls but unaffected during pregnancy. The F-value was 65.00 (p=0.000) statistically significant. The mean protein level decreased in 3rd and 2nd trimesters in pregnant respondents as compared to 1st as well as non-pregnant healthy women and it was found to be statistically significant with F-value 32.00 (p=0.000).

Table 4: Protein Profile of participants

Protein Profile (gm/dl)	Pregnant Women (Trimesters) (n=224)			Non Pregnant Healthy Women (n=112)	F-value	p-Value
	1st	2nd	3rd			
Albumin	4.3±0.54	4.0±0.49	3.9±0.52	4.8±0.52	62.4	0.000
Globulin	2.7±0.37	2.6±0.34	2.5±0.30	2.4±0.26	65.00	0.000
Protein	7.0± 0.62	6.6±0.56	6.4±0.54	7.2±0.62	32.00	0.000

3.6 Correlation between BMI and Liver function Profile

The correlation between BMI and ALP was positively correlated and highly significant (r=0. 239; p<0.01). However, the relationship between ALT and AST with BMI was insignificant as shown in Table 5.

Table 5: Correlation between BMI and Liver function Profile (N=336)

		BMI	ALT	AST	ALP
BMI	Pearson Correlation	1	-	-	-
	Sig. (2-tailed)	-	-	-	-
ALT	Pearson Correlation	-0.054	1	-	-
	Sig. (2-tailed)	0.321	-	-	-
AST	Pearson Correlation	0.051	0.502**	1	-
	Sig. (2-tailed)	0.348	0.000	-	-
ALP	Pearson Correlation	0.239**	0.053	0.238**	1
	Sig. (2-tailed)	0.000	0.337	0.000	-
**. Correlation is significant at the 0.01 level (2-tailed).					

3.7 Correlation between BMI and Protein Profile

There was negative correlation between albumin, total protein with BMI and highly significant (r= -0.268; p<0.01; r= -0.215; p<0.01) respectively. However, the relationship between BMI and globulin was not significant as illustrated in Table 6.

Table 6: Correlation between BMI and Protein Profile (N=336)

		BMI	Globulin	Albumin	Total Protein
BMI	Pearson Correlation	1	-	-	-
	Sig. (2-tailed)	-	-	-	-
Globulin	Pearson Correlation	0.008	1	-	-
	Sig. (2-tailed)	0.888	-	-	-
Albumin	Pearson Correlation	-0.268**	-0.272**	1	-
	Sig. (2-tailed)	0.000	0.000	-	-
Total Protein	Pearson Correlation	-0.215**	0.341**	0.751**	1
	Sig. (2-tailed)	0.000	0.000	0.000	-
**. Correlation is significant at the 0.01 level (2-tailed).					

4. Discussion

Age is the single biggest factor affecting a woman's fertility and is related to ovulation, menstruation and menopause. The highest numbers of pregnant and non-pregnant women were in between 20-30 years and lowest in between 30-40 years. Age group between pregnant and non-pregnant healthy women was found to be statistically insignificant (p = 0.4332). Azab *et al* found that 48.4% pregnant women between 21-30 years old, 40.8% were with age between 31-40 years, 5.8% were with age >40 years, and 5% were with age <20 years, [29] which are not in accord with this study. Similarly, the ages of the pregnant women in Derna city, Libya was ranged from 15 to 48 years with a mean age of 30.3±6.2 years. [30]

Akinbami *et al* reported that the mean age of pregnant women in Lagos, Nigeria was 30.52±4.6 years (range: 20–46 years old). [31] But, the mean age of pregnant women in Northwestern Ethiopia was 26.13 year±4.55 year standard deviation. The minimum and maximum age of the study participants were 17 and 45 years, respectively. Melku and Agmas recorded that 38.2% of pregnant women were in between 26-30 years old, 37.4% were with age between 20-25 years, 12.3% were with age <20 years, 8.6% were with age between 31-35 years, and 3% were with age >35. [32]

The beginning of their reproductive years is marked by the onset of ovulation and menstruation. The 20-30 years is the most fertile years where periods are probably regular and ovulatory chances are more probable. The possibility of being more pregnant in this age group is that the average women in this age group have about 20 percent chance each month of getting pregnant when unprotected intercourse occurs. Also, a woman at this age is likely to be focused more on her marriage than on other parts of her life.

Other possible reasons may be the desires of their partner, family and comfort years to be pregnant. An easier delivery and able to get the body back in shape more quickly in this age group may be the one of the other prospect. The lowest pregnancy in between 30-40 years may be due to the fact that fertility begins to decline at or after at the age of 30 but this change happens gradually, over the next five years or so. An almost linear downward trend is observed after age 35. [33] The risk of high blood pressure during pregnancy is about double for women over 35 compared with younger ones; hypertension affects about 10 to 20 percent of pregnant women in this age group. [34]

The present study illustrates of total 336, 33% were non-pregnant healthy women and 67% pregnant women where 17%, 26% and 24% were in 1st, 2nd and 3rd trimester of pregnancy. The same type of study conducted by Michael *et al* found that 83.4% of 253 were pregnant with 10.9% in 1st trimester, 46.0% in second trimester and 43.1% in 3rd trimester and 16.6% were non-pregnant control [35] which is almost similar to this study. Both studies highlight more number of study participants were enrolled in 2nd trimester. This may be due to the most of the participants might be from the local area surroundings of study setting.

In the present study, women with multi gravidity and multiparity constituted higher (61.61%) and 70.99% respectively. There is little work currently available examining the reasons for the gravidity and parity, particularly in communities with high gravid and parity, however one included in this study, in which two third (77.0%) of the women were multigravid and multiparous [29] which is concurrent with this study.

Maternal anthropometry differs across populations. [36] Nutrient intake and weight gain during pregnancy are the two main modifiable factors influencing maternal and infant outcomes. [37] The study highlights that weight and BMI in pregnant women increases with the trimesters of pregnancy and was statistically significant (p= 0.000). But, the association of height between the pregnant and non-pregnant was statistically insignificant (p= 0.545).

The rise of BMI advanced with pregnancy was also confirmed by Geraghty *et al* and Jin *et al* and is parallel to current study. [38, 39] Gestational weight gain, hormonal alteration and high diet may be the contributing factor for increasing BMI**.** Normal pre-pregnancy BMI is protective for adverse pregnancy and neonatal outcomes and has been associated with less obstetrical interventions. [40] Indeed, a low body mass index (BMI) and suboptimal weight gain during pregnancy are long-recognized risk factors for the delivery of infants too small for gestational age. [41]

During pregnancy, the serum estrogen and progesterone levels increase progressively and reach a maximum during the third trimester. These sex steroids have effects on metabolic, synthetic, and excretory hepatic functions. [42] Histological evaluation of liver biopsies, including examination with electron microscope, has shown no distinct changes in liver morphology in normal pregnant women. But, some of the laboratory tests used to evaluate hepatic function yield appreciably different results during normal pregnancy. Moreover, some of the changes are similar to those in non-pregnant patients with liver disease. [43]

Liver cells contain several enzymes which may be released into circulation in liver damage. Measurement of aminotransferases group of enzymes in serum namely serum glutamate pyruvate transaminase (SGPT; recently called as Alanine transaminase-ALT) and serum glutamate oxaloacetate transaminase (SGOT; recently known as Aspartate transaminase-AST) are widely used to assess the liver function. The present study reports mean ALT level with standard deviation of participants in their 1st, 2nd and 3rd trimester were 15.20±9.14, 12.32±5.89, 11.20±6.07 respectively and the ALT level in healthy non-pregnant subjects was 13.80±4.60. The mean ALT level was decreased in the 2nd and 3rd trimester as compared to 1st as well as non pregnant healthy women.

Loganathan *et al* also confirmed ALT levels were lower in pregnant mothers which is accord with this study [44]. A study conducted by Al-Tawil of Islamic University in Gaza [45] also noted the same results. Larrain and Girling highlights that healthy pregnant women typically do not have elevated aminotransferases and, in fact, levels may decrease in pregnancy. [46,47] However, in normal pregnancy aminotransferases can rise in the puerperium. Non-alcoholic fatty liver disease (NAFLD) is the most

common cause of abnormal aminotransferases and also seen in pregnancy [48]. But, in contrast, Bacq *et al* concluded that serum ALT activity was significantly higher during second trimester of pregnancy compared with non pregnant women. An increase in ALT levels was found during labour, which might be caused by contraction of uterine muscle. [49]

Similarly, a study carried out between March 2012 to April 2013 at B. J. Medical College and Civil Hospital, Ahmedabad, India estimated ALT levels as 23.54±11.92; 23.42±8.68; 34.22±23.57 and 22.48±4.40 in 1st, 2nd and 3rd trimesters and in control respectively. [3] Serum ALT activity was significantly higher during third trimester compared to first trimester and controls. The results are not in accord with present study. Pradumna *et al*., Joshi *et al*., and Bacq *et al* demonstrated ALT activity levels do not change during pregnancy or remain within the normal limits established in non pregnant women [9,21,49] which diverges with current study.

The present study reveals mean AST level with standard deviation 18.50±7.99, 18.52±6.00 and 18.53±6.59 in their 1st, 2nd and 3rd trimesters respectively and 19.22±6.43 in healthy controls. The AST levels were found as 23.31±18.18; 23.88±14.99; 34.92±26.94; and 20.00±5.54 in 1st, 2nd and 3rd trimesters and in control respectively in a study carried by Gohel *et al* [3]. The results are parallel to present study. In the majority of published literatures, AST activity levels do not change during pregnancy or remain within the normal limits established in non pregnant women [21, 50, 51] which is alike with present findings.

Salman, at Beladi laboratoty of Al-Ramadi General Hospital for Maternity and Children analyzed blood for AST activity reported no significant difference in serum AST between pregnant and non-pregnant women [52] which is almost parallel to this study. Also, Bacq *et al* found serum AST activity was not significantly different in pregnant and non pregnant women [49] which are analogous to results of current study. But, Loganathan *et al* found AST level lower in pregnant women [44] which do not match with the present results. On the other hand, elevated serum aminotransferases may also be associated with raised serum ammonia, amino acid levels and lactic acidosis, uric acid, hyperbilirubinemia and hypoglycemia secondary to impaired hepatic glycogenolysis. [53]

Alkaline phosphatases are membrane-bound glycoproteins found in many tissues. [54] ALP originates mainly from two sources: liver and bone. There are four main alkaline phosphatase variants, intestinal, placental, placental-like and a non-tissue specific isoform. The enzymes may be present in other tissue like intestine, kidney, placenta, leukocytes. The serum ALP values rises in third trimester. [9] This study revealed mean ALP level with standard deviation in control was 105.58±15.88 and in cases were 72.32±21.38, 79.57±15.37and 126.97±15.97 in their 1st, 2nd and 3rd trimester of pregnancy. Serum ALP activity was significantly higher in third trimester compared with non-pregnant, first and second trimester pregnant women. Another cross sectional study carried over a period of one year on 150 pregnant women and 50 age matched control depicted ALP level as 152.5±49.5; 195.8±34.7; 399.1±147.4 in 1st, 2nd and 3rd trimesters and in control respectively. [3] The increased ALP level is in correspondence to current study.

The similar results were also reported in earlier studies carried out by Joshi *et al*., Loganathan *et al* and Jamjute *et al* [21,44,51] which is compatible to present study. The raise in ALP is mainly attributed to the added placental secretion or due to increase in the production of the bone isoenzymes. [21,50] Because of the lack of specificity, the measurement of serum ALP activity is a poor test for the diagnosis of cholestasis during the third trimester of pregnancy. [43] Alkaline phosphatase may be elevated up to 10 times normal; however, alkaline phosphatase can also be increased during the third trimester normally. [55] Bacq *et al* also confirmed serum ALP activity significantly higher during the third trimester compared to non-pregnant women and during second trimester compared to first trimester [49] is quite similar with current study. Same type of findings was also experienced by Salman for ALP activity during his research. [52]

Most studies showed increase in ALP activity in second and third trimester. [56, 57] Alkaline phosphatase activity in the placenta is associated with preterm delivery [58] and trophoblast alkaline phosphatase activity is altered in pre-eclampsia. [59] The regulation of human placental alkaline phosphatase is not well described, but is transcriptionally regulated by steroid hormones, peroxisome proliferator-activated receptor (PPAR-γ) [60] and the short chain fatty acid butyrate. [61-63]

Albumin is synthesized in the liver. The rate of synthesis is constant in normal individuals at 150 to 250 mg/kg/day, resulting in the production of 10 to 18 g of albumin daily in a 70-kg man. The liver produces albumin at less than half of its capacity. The primary factors affecting albumin synthesis include protein and amino acid nutrition, colloidal osmotic pressure, the action of certain hormones, and disease states. [64] A considerable amount of albumin passes through the glomerular filtrate daily during pregnancy and most of this is reabsorbed in the renal tubules, being broken down in the process and therefore lost to the body. Moreover, albumin is the major protein frequently found in normal urine. [65,66] The present study reports the mean albumin level (gm/dl) with standard deviation in pregnant women in their 1st, 2nd and 3rd trimester were 4.3±0.54, 4.0±0.49, 3.9±0.52 respectively and the albumin level in non-pregnant healthy women was 4.8±0.52. This finding demonstrates the mean albumin level

was decreased in the 2nd and 3rd trimester as compared to 1st as well as non pregnant healthy women (p<0.000).

A study carried by Noor *et al* at Dhaka Central International Medical College, Dhaka estimated serum albumin (g/dl) as 3.97±0.64; 3.53±0.53 and 3.53±0.54 in 1st, 2nd and 3rd trimesters respectively and in non pregnant healthy women as 4.30±0.71. [67] The results are quite similar to current study. Similarly, the study carried by Zannat *et al* determined serum albumin of control group of non-pregnant women in reproductive age and women in first and third trimester of pregnancy were 3.83±0.027, 3.57±0.044 and 3.31±0.05 respectively. Serum albumin was decreased in first and third trimester of pregnancy than in non pregnant women and maximum decrease seen in third trimester. [68] The results obtained confirms with present study. Maryam *et al* showed serum albumin level decreased from the first trimester and became progressively more accentuated with the advancement of pregnancy. [69] The results of present study also bears similarity with the studies of Bacq *et al* conducted on pregnant women in France. [49] Similar results parallel to current study were also obtained by Gohel *et al* [3].

Correspondingly, Bagga *et al* concluded that mean value of serum albumin concentration before 20 weeks of pregnancy were lower in normal group. [70] The results are in accordance with present study. Honger claimed that the synthesis of albumin is inhibited by progesterone and or estrogen. [71] On the other hand, Mendenhall demonstrated that gestation is associated with a decrease in albumin concentration with no variation in serum immunoglobulins. [72] This may be due to increase in plasma volume approximately 50% from the 6th to 36th weeks of gestation which lead to hemodilution. This lower concentration of serum albumin is claimed to be result of proteinuria and hyper catabolism of albumin with no detectable loss of albumin in interstitial fluid or gut. In addition, gradual decrease in total protein and albumin levels were also observed in different trimesters of pregnancy reported by Noor *et al* [67] is likely to present study.

The findings of previous investigators Haram *et al* & Ekeke *et al* [73,74] are in line with the current study. The extent of the fall in this study is much higher than the fall in studies carried out on subjects from economically advanced countries. Nutritional deficiencies may be implicated for this phenomenon. [75-77] On the other hand, Olufemi *et al* found that the amount of albumin synthesized in the intravascular compartment was significantly greater at 9.5 g/day in pregnant subjects compared with 6.3 g/day in non-pregnant control subjects which is contradictory to the existing results [78] .

Globulins are a heterogeneous group of large serum proteins other than albumin which includes hundreds of serum proteins including carrier proteins, enzymes, complement, acute phase proteins, lipoproteins and immunoglobulins. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. [64] The current study reports that the mean globulin level with standard deviation was 2.7±0.37, 2.6±0.34 and 2.5±0.30 in their 1st, 2nd and 3rd trimesters respectively and 2.4±0.26 in healthy controls. The result depicts minor variation in different trimesters as compared to controls. The F-value was 65.00 (p<0.000) statistically significant. Similarly, in another study serum globulin (g/dl) in pregnant females was 2.64±0.75; 2.78±0.77; 2.49±0.70 in 1st, 2nd and 3rd trimesters respectively and in non pregnant women as 2.61±0.88 [67]. The results are in accord with present study.

The almost stable serum globulin in the face of haemodilution suggests an increased serum globulin content of blood during pregnancy. Increases in the globulin fraction usually result from an increase in immunoglobulins, but there can be an increase in other proteins in pathologic states that have characteristic electrophoretic patterns. Malnutrition and congenital immune deficiency can cause a decrease in total globulins due to decreased synthesis, and nephrotic syndrome can cause a decrease due to protein loss through the kidney. [64]

Delivery has been associated with increased dietary protein requirements in humans. During their period of rapid growth, the foetus and placenta accrue proteins very rapidly. [79] Protein status is usually assessed by measuring levels of total serum proteins, albumin, or plasma non-essential and essential amino acid ratio. [80] It is universally known that serum protein anabolism occurs in the liver and plasma cells and catabolism of low molecular weight in the kidneys. Alterations in function of any of these sites will affect the appropriate serum protein fractions. [19] The present study depicts the mean protein level with standard deviation in non-pregnant healthy women was 7.2±0.62 and the figures in the pregnant women were 7.0±0.62, 6.6±0.56 and 6.4±0.54 in their 1st, 2nd and 3rd trimester of pregnancy. This reveals that the mean protein level decreased in 2nd and 3rd trimesters in pregnant respondents as compared to 1st as well as non-pregnant healthy women and it was found to be statistically significant F-value 32.00 (p<0.000).

Likewise, a study carried at Dhaka, Bangladesh noted total protein (gm/dl) as 6.61±1.00; 6.31±1.02 and 6.01±0.94 in 1st, 2nd and 3rd trimesters respectively and in non-pregnant healthy women as 6.92±0.98. [67] The results are in accordance with present study. Another study carried by Adedeji *et al* highlights progressive fall in total protein was at 33-36 weeks of gestation. [81]

The total serum protein concentration is known to be of limited value on its own. An important factor in the etiology of protein changes is the effect of hormones, especially estrogen, on the synthesis and degradation of

proteins such as alpha-fetoprotein, salivary amylase, prolactin and the proteins of the "pregnancy zone". [82]

The explanatory clarification towards decreased serum protein level is that the increase in plasma volume that occurs during pregnancy leads to haemodilution. Hence, it decreases the serum protein concentrations without altering the albumin: globulin ratio. The changes in total serum protein concentration may result from dehydration and over loading with fluid.

The principles of serum protein changes are lowering of the concentration of albumin fraction probably resulting from renal serum albumin loss, which may have exceeded the reported increase in serum albumin synthesis. A direct relationship of quality and quantity of dietary proteins with decrease in plasma proteins in cases of protein malnutrition has been reported and maternal malnutrition may be aggravated by pregnancy. [83] It has been suggested that pregnant women in developing countries consume diets with a lower quantity of protein, minerals and vitamins. [84] This inadequate dietary intake may be responsible for hypoproteinemia and hypoalbuminemia during pregnancy. [85]

There was positive correlation between BMI and ALP and found to be highly significant ($r=0.239$; $p<0.01$). This may be due to the fact that gestational weight gain may lead to maternal obesity which could alter plasma non-esterified fatty acids (NEFA) profiles regulating alkaline phosphatase expression via peroxisome proliferator-activated receptors (PPARs) or histone deacetylases (HDACs). [60]

Corticosteroids and oestrogen could also regulate alkaline phosphatase expression and differences in the levels of these hormones, their binding proteins or their placental receptors as a result of maternal obesity. [63,86] Alternatively, it may not be the maternal obesity itself, but an obesogenic diet or lifestyle that alters regulation of placental alkaline phosphatase. The other justification might be the strong associations between the expression of placental alkaline phosphatase and other lipid-related genes, including leptin, which was positively related to placental alkaline phosphatase expression. [87]

Conversely, the relationship between ALT with BMI was negatively correlated and insignificant. Also, there was no significant relation between AST and BMI. For protein profile, association between albumin, total protein and BMI was found to be negatively correlated but highly significant ($r= -0.268$; $p<0.01$; $r= -0.215$; $p<0.01$) respectively. This might be due to increasing permeability and distribution volume in the course of pregnancy. It has been suggested that low Body Mass Index (BMI) is associated with a decrease in serum protein levels predisposing them to other illnesses. [88] However, the relationship between BMI and globulin was not significant. Other biochemical parameters like albumin and ALT is mainly produced from hepatocytes and in case of any infection or inflammation its serum level exceeds normal range. Thus, it is regarded as a well-known marker of liver infection. [89] However, AST is not a better predictor of hepatocytes injuries, because it is produced from various body organs such as liver, kidney, lung, brain and placenta. Serum levels of albumin characterize hepatocytes functionality and biliary obstruction respectively. No increase in ALT, AST and other liver function related biomarkers somehow exclude the liver for the release of ALP and thus support its origin from adipose tissue. [88-90]

5. Conclusion

Physiologic changes that occur in every organ system during pregnancy cause alteration in normal laboratory values associated with hepatic function. The liver enzyme especially serum ALT level was decreased during 3rd and 2nd trimesters whereas level of AST signifies small variation in different trimesters as compared to 1st trimester and non pregnant healthy women. The correlation between ALT and AST with BMI was insignificant. The ALP levels was drastically increased in 3rd trimester and moderately increased in 2nd trimester as compared to controls. The relationship between BMI and ALP was positively correlated and was highly significant. Small variation was depicted in levels of serum albumin, globulin and total protein during pregnancy. There was negative correlation between albumin and total protein with BMI found to be highly significant. On the other hand, the relationship between globulin and BMI was insignificant. Unless gestation related alterations in LFTs values are taken into account in a pregnant woman, physiologic adaptations of pregnancy can be misinterpreted as pathologic or, alternatively, pathologic findings may not be recognized. Proper evaluation and diagnosis that may help in appropriate obstetric planning with regards to the timing of delivery in preventing maternal and fetal complications.

Limitation of the study

Some of the markers of liver function tests were not included due to shortage of time period. The majorities of participants were from surroundings of study area and cannot be related to the whole population of Province No. 2 in Nepal.

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Conflict of Interest

Authors declared that there is no conflict of interest.

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