Original article:

Evaluation of liver function tests in different trimesters of normal pregnancy

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Abstract:

Introduction: Pregnancy is a physiological condition which brings about changes in different systems of the body to support the growing fetus in the uterus. The increase levels of sex hormones estradiol and progesterone during normal pregnancy effect the hepatic metabolism, synthesis and excretory function.

Aim: This study was designed to evaluate some of the liver function tests in different trimesters of pregnancy and to study the variations taking place in normal pregnancy.

Method and materials: In this study 180 pregnant women, 60 in each trimester all attending antenatal OPD in Jorhat Medical College, Jorhat are taken. They were compared with 60 non pregnant healthy women without oral contraceptives. The liver function tests comprising of total Protein, Albumin, total bilirubin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma glutamyl transferase (GGT) and ALP (Alkaline phosphatase) were estimated in dry chemistry autoanalyser Johnson & Johnson's Vitreous 250. The statistical analysis was done by using student 't' test.

Results: From the study it was found that the serum albumin values decreased in all the trimesters of pregnancy when compared with non pregnant control group, $P < 0.001$.Total bilirubin values also show a lower trend when compared with control group,$P < 0.05$. The ALT values decreased in 3rd trimester $P < 0.001$ when compared with control group.. The ALP values increased significantly in all the trimesters when compared with the non pregnant control group, $P < 0.001$

Conclusion: There is decrease in total protein and albumin and increase in ALP in normal pregnancy which should be correlated clinically before terming them to be pathological.

Keywords: Alanine aminotransferase, Aspartate aminotransferase, Alkaline Phosphatase

Introduction:

Pregnancy is a physiological condition which brings physical change in the reproductive age of a woman. From the time of conception the reproductive system of a woman changes through the three trimesters of pregnancy. Besides the reproductive system other systems of the body such as the renal, endocrine, nervous, cardiovascular, respiratory, gastrointestinal system and hepatobiliary system are also affected. The levels of estrogens (estradiol) and progesterone increase progressively during pregnancy[1,2].

These sex hormones have effects on hepatic metabolic, synthesis, and excretory functions[3,4,5]. The biliary excretion of bromosulfophthalein decreases during late pregnancy and the clearance of some compounds that are secreted into bile may therefore be impaired[6]. The liver has a central and critical biochemical role in the metabolism, digestion,

detoxification and elimination of substances from the body[7].

The liver receives, processes and stores amino acids, carbohydrates, lipids, vitamins and minerals. In addition, many of the proteins including alpha and beta globulins, coagulation factors and transport proteins are synthesized. The liver is also the primary site of detoxification of exogenous compounds such as drugs and toxins[8]. Another major function is the conjugation of bilirubin with glucuronic acid to produce bilirubin monoglucoronide and diglucuronide which are then excreted into the bile thus regulating cholesterol metabolism and fecilitating the absorption of dietary fat. The liver is a major site for catabolism of thyroid, steroid and other hormones and thereby helps regulate plasma hormone levels[9]. Liver functions are assessed by measuring the concentration of substances produced by hepatocytes or by measuring the serum content of substances released from these cells as a result of damage and assessing the capability of liver to perform metabolic functions like detoxification, conjugation[10]. Serum aminotransferases assays are the most common laboratory tests for detection of liver diseases and these include aspartate aminotransferase (AST) and alanine aminotransferase (ALT). They are excellent markers of hepatocellular injury[11]. AST is found primarily in the heart, liver, skeletal muscles, kidney, brain, pancreas, lungs, leucocytes and red blood cells[12] while ALT is found primarily in the liver, kidney, with lesser amount in the heart and skeletal muscles [13,14]. ALT is thought to be more specific for hepatic injury because it is present mainly in the cytosol of the liver cells and in low concentration elsewhere[15] while AST is both cytosolic (20% of total activity) and mitochondria (80% of total activity), it is less sensitive and specific for the liver[16]. Transaminase reactions are monitored continuously by coupling of the transaminase reactions to specific dehydrogenase reactions. The oxaloacetate formed in the AST reactions is reduced to malate in the presence of malate dehydrogenase and pyruvate formed in the ALT reaction is reduced to lactate by the action of lactate dehydrogenase. Alkaline phosphatase is present in practically all tissues of the body, especially at or in cell membranes and it occurs in particularly high levels in intestinal epithelium, kidney tubules, bones (osteoblasts), liver and placenta[17]. It catalyzes the hydrolysis of phosphate esters in alkaline environment generating an organic radical and inorganic phosphate[18]. Hepatobiliary disease and bone disease associated with increased osteoblastic activity are linked to increased serum alkaline phosphatase. Gamma glutamyl transferase is present in all cells except those in muscles. Some enzyme is present in cytosol, but the larger fraction is located in the cell membrane and may transport amino acids and peptides into the cell across the cell membrane in the form gamma glutamyl peptides[17].GGT activity is highest in cases of intrahepatic or posthepatic biliary obstruction, reaching levels some 5 to 30 times normal. It is also elevated in primary or secondary neoplasms, pancreatitis, fatty liver, alcoholic cirrhosis and also chronic alcoholics. Bilirubin is the final product of heme degradation[19]. The iron porphyrin or heme group of hemoglobin is released from dying erythrocytes in the spleen is degraded to yield free Fe^{++} and ultimately bilirubin a linear(open) tetrapyrole derivative. Bilirubin bind to serum albumin and is transported to the liver where it is transformed into the bile pigment bilirubin glucuronide which is sufficiently water soluble to be secreted with other components of bile into the small

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intestine. For each mole of heme catabolized one mole of carbon monoxide, bilirubin and Fe^{2+} are produced. Daily production of bilirubin in human averages 250 to 300mg approximately[20].Because the unconjugated bilirubin is toxic and insoluble it must be glucuronited before excreted into the bile[21]. Impaired liver function or blocked bile secretion causes bilirubin to leak into the blood resulting in jaundice[22]. Most plasma proteins originates in the liver, reticuloendothelial cells, plasma cells, general tissue cells and disintegrating blood cells. The total plasma concentrations normally ranges from 60 to 80gm/l. Albumin ranges from 35 to 50gm/l and globulin from 20 to 35gm/l[23]. Total protein may be altered by changes in plasma volume, an increase caused by dehydration and a decrease from overloading with water. In chronic hepatocellular damage there is impaired albumin synthesis with an accompanying fall in serum albumin. Albumin measurements provide a fairly good index of the progress of chronic disease but in acute liver disease however there may be little or no reduction in serum(albumin) as the biological half life of albumin is about 20 days and fractional clearance rate is therefore low[24].

Aim of the study: The study has been proposed to see the status of liver function during normal pregnancy and to rule out subclinical liver disorders if any.

Materials and methods: This study was conducted in Jorhat Medical College, Jorhat, Assam.

Study design: It is a hospital based case control study.

Study population: Pregnant women of different trimester attending antenatal outpatient department in obstetrics and gynecological department of Jorhat Medical College.

Sample size: 240, out of which 60 cases are in the 1st trimester, 60 are in the second trimester, 60 cases in the 3rd trimester and 60 normal healthy non pregnant women without taking oral contraceptives as control group.

Study duration: From May 2015 to October 2015(six months).

Inclusion criteria:

i. All antenatal cases between 18 to 40 years of age
ii. Spontaneous conception
iii. Singleton pregnancy
iv. No history of hypertension, diabetes or liver disease
v. No history of intake of hepatotoxic drugs

Exclusion criteria:

i. Known liver disease
ii. Hypertensive patients
iii. Assisted conception
iv. Multiple pregnancy

Methodology: Detail history and written consent was taken from the patient before recruiting them.

Procedure: With all aseptic and antiseptic measures 5 ml of blood is collected from the central median cubital vein from both control and experimental groups. The blood is immediately transferred into labeled clotted vials after removing the needle very slowly to prevent hemolysis. Then the blood is allowed to clot for 30 to 45 minutes after putting the stopper to the vial. The clot is rimmed and centrifuged for 5 minutes at 3000 rpm in a clinical centrifuge machine. All the parameters – AST, ALT, ALP, GGT, Total protein, Albumin and Total bilirubin were estimated in vitreous 250 autoanalyser by reflection spectrophotometry using dry slide from orthoclinical diagnostic (part of Johnson and Johnson Company USA).

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Statistical analysis: The results were expressed as mean± standard deviation. The data analysis was carried out using student "t" test[25]. Comparison of parameters between normal nonpregnant women (control) and pregnant women in different trimesters was done with student 't' test (paired "t" test). A 'P' value < 0.05, P < 0.01 and P < 0.001 were considered statistically significant.

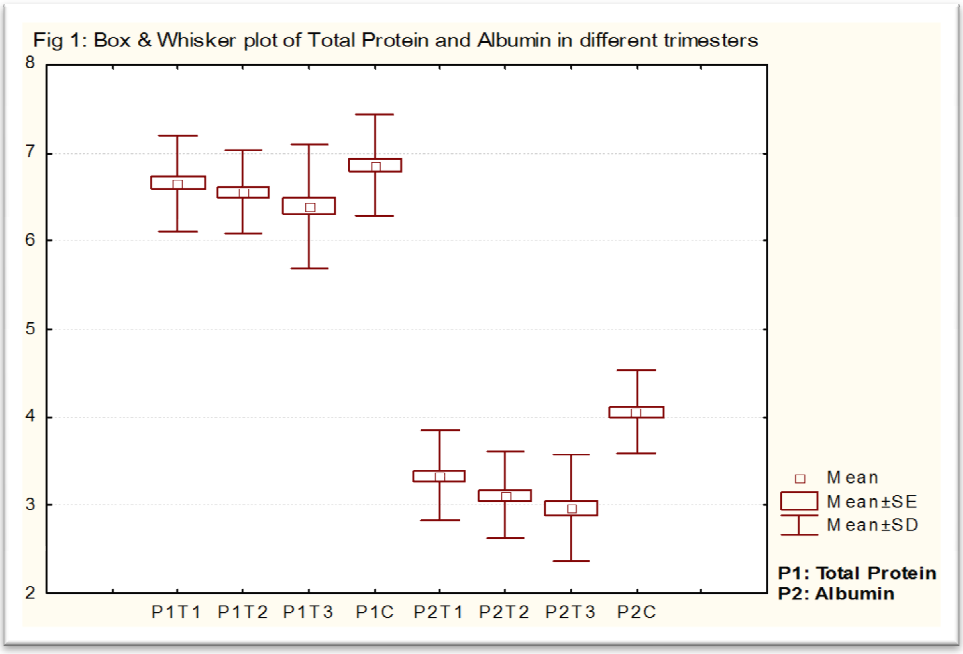
Results:

In this study 180 normal pregnant women were taken as cases out of which 60 in the 1st trimester, 60 in the 2nd trimester and 60 in the 3rd trimester attending antenatal OPD. They were compared with 60 healthy non pregnant women without oral contraceptives.

Results of the study are given in table: 1, table: 2 and table: 3. In table: 1, the mean values ±SD of total protein shows a decreasing trend in the three trimesters of pregnancy.

TABLE 1: Statistical Analysis of T.Protein, Albumin & T.Bilirubin in different trimesters

	Total Protein				Albumin				T.Bilirubin			
	CG	*T1*	*T2*	*T3*	*CG*	*T1*	*T2*	*T3*	*CG*	*T1*	*T2*	*T3*
Mean ± SD	6.86 ±0.57	6.66 ±0.54	6.56 ±0.47	6.39 ±0.70	4.06 ±0.48	3.33 ±0.52	3.11 ±0.49	2.97 ±0.61	0.92 ±0.12	0.78 ±0.44	0.81 ±0.33	0.82 ±0.31
SE	0.07	0.07	0.06	0.09	0.06	0.07	0.06	0.08	0.01	0.06	0.04	0.04
Min - Max	5.9-7.9	5.6-8.0	5.2-7.4	4.1-7.5	3.2-4.9	2.1-5.6	1.7-4.2	1.1-3.9	0.6-1.0	0.2-3.0	0.3-2.0	0.32-1.83



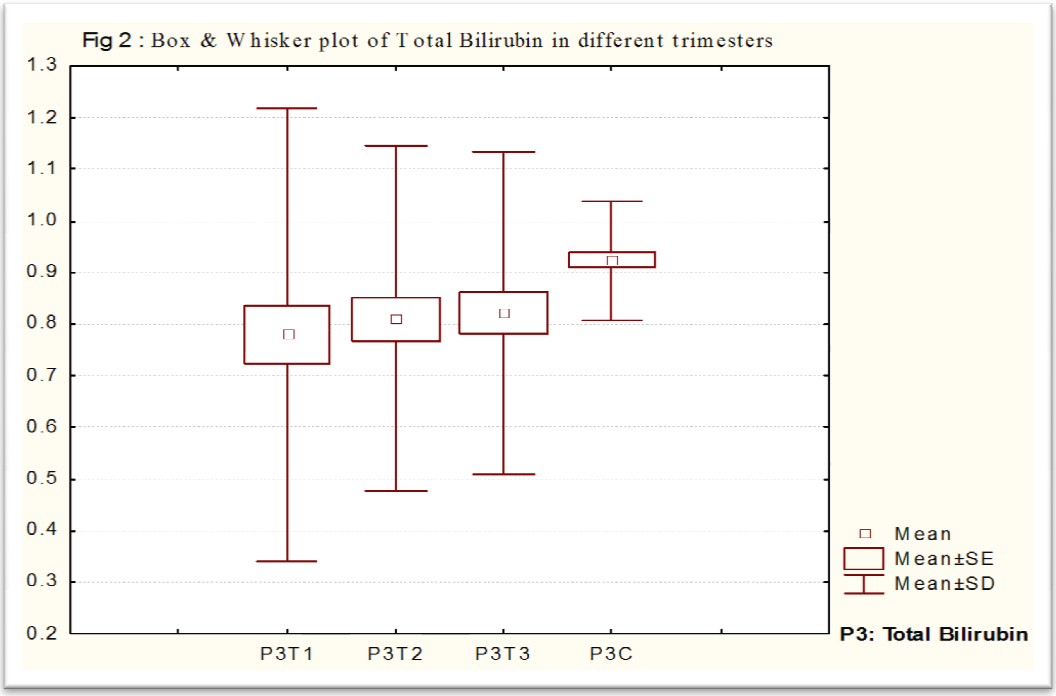
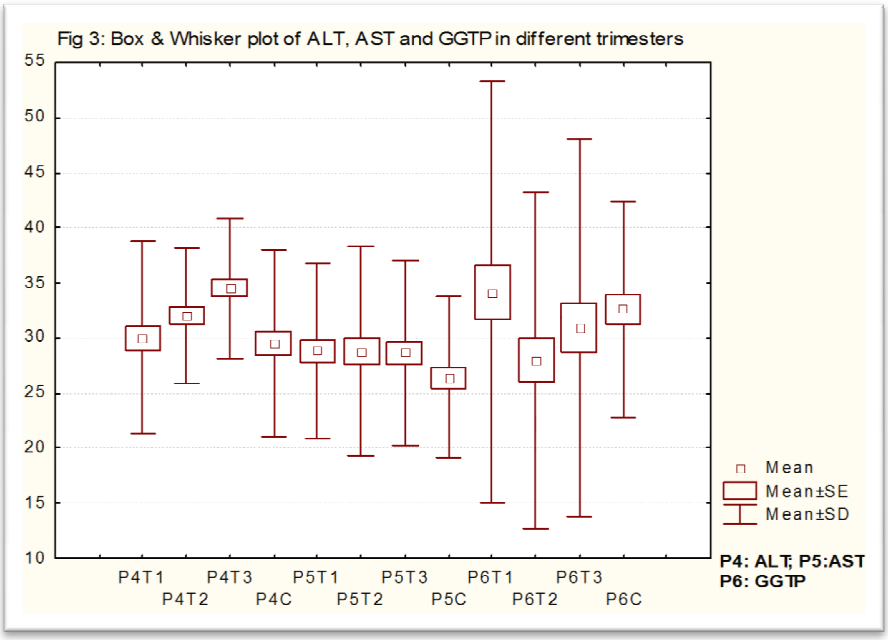


TABLE 2: Statistical Analysis of AST, ALT, GGTP & ALP in different trimesters

	ALT				AST				GGTP				ALP			
	CG	*T1*	*T2*	*T3*	*CG*	*T1*	*T2*	*T3*	*CG*	*T1*	*T2*	*T3*	*CG*	*T1*	*T2*	*T3*
Mean ± SD	29.57 ±8.47	30.02 ±8.71	32.03 ±6.08	34.52 ±6.34	26.43 ±7.34	28.83 ±7.93	28.78 ±9.47	28.67 ±8.37	32.60 ±9.88	34.13 ±19.1	27.97± 15.27	30.93± 17.19	78.73 ±28.9	90.60 ±42.7	167.00 ±52.13	305.12 ±75.69
SE	1.09	1.12	0.78	0.82	0.95	1.02	1.22	1.08	1.28	2.47	1.97	2.22	3.73	5.51	6.73	9.77
Min - Max	12 - 45	12 - 44	20 - 47	24 - 49	16 - 45	10 - 46	18 - 71	12 - 46	14 - 56	10 - 96	10 -98	16 - 141	34 - 148	28 - 200	42 - 355	108 - 460



t-Test: (T<=t) two-tail(Paired Two Sample for Means); t Critical two-tail: 2.000

SD: Standard Deviation, SE: Standard Error, CG: Control group, T1: 1st trimester, T2: 2nd trimester, T3: 3rd trimester

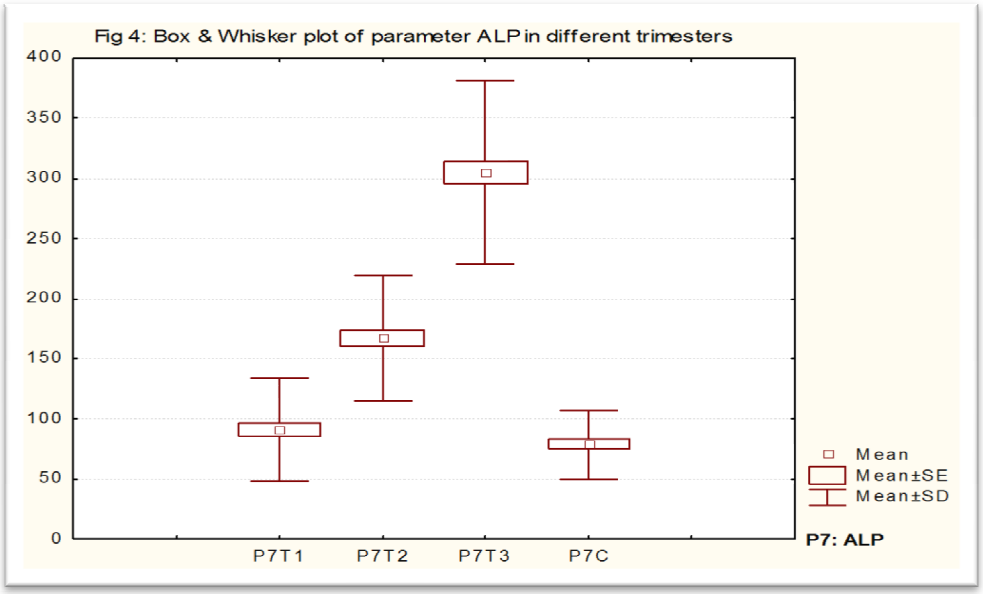


TABLE 3: P & t values when (compared with CG) for different parameters

Parameter		Total protein	Albumin	ALT	AST	ALP	GGTP	T.Bil
T1	P	0.031	<0.001	0.758	0.064	0.043	0.561	0.021
	t	2.209	8.720	-0.309	-1.89	-2.066	-0.584	2.374
T2	P	0.003	<0.001	0.077	0.121	<0.001	0.061	0.014
	t	3.107	10.250	-1.799	-1.574	-10.720	1.906	2.522
T3	P	0.001	<0.001	0.001	0.126	<0.001	0.538	0.020
	t	3.640	10.940	-3.606	-1.552	-21.470	0.619	2.394

 # t-Test: (T<=t) two-tail (Paired Two Sample for Means); t Critical two-tail: 2.00; SD: Standard Deviation,

 # SE: Standard Error, CG: Control group, T1: 1st trimester, T2: 2nd trimester, T3: 3rd trimester

The P value becomes highly significant ie $P \leq 0.001$ in the 3rd trimester. The mean values ± SD of serum albumin has decreased in all the trimesters when compared with normal control (non pregnant) group P, ≤ 0.001 highly significant and t value increasing to 10.94 in the 3rd trimester. The mean values with SD and SE for T Protein and albumin are shown in figure 1. The mean values ± SD of total bilirubin is less in the three trimester of pregnancies and it is significant , $P \leq 0.05$ but within the normal range. The mean values with SD and SE of total bilirubin are shown in figure 2. In table 2, the mean values ± SD of ALT shows an increasing trend in 1st , 2nd and 3rd trimester of pregnancies. The P value is insignificant in 1st and 2nd trimesters but becomes significant 3rd trimester with P value ≤ 0.001. It is well plotted in figure 3 with SE 0.82 in the 3rd trimester. The mean values ± SD of AST does not show much difference when compared with the control non pregnant group. The P value is insignificant, $P > 0.05$ all throughout the trimesters and t value closure to 0. The mean values ± SD of GGT also does not show much difference throughout different trimesters when compared with normal control non pregnant group, $P > 0.05$ in all the trimesters. The mean values with SD and SE are well plotted in figure 3. In table 2 the mean values ± SD of ALP shows a significant increase in all trimester especially 2nd and 3rd trimesters where $P < 0.001$ and t value is -10.72 and -21.47 respectively. The mean values with SD and SE are well plotted in figure 4.

Discussion:

In this study, the total protein is seen to decrease gradually in the experimental groups (1st, 2nd and 3rd trimesters) when compared with the non pregnant control group. It is highly significant $P<0.001$ in 3rd trimester. The plasma volume increases steadily from the 6th to the 36th week of gestation by about 50% but the increase in red blood cells in moderate (about 20%) and delayed. The total protein decreases due to this haemodilution. From table 1 and figure 1, it is seen serum albumin decreases significantly in all the trimesters when compared with control group, $P < 0.001$.Serum albumin levels decrease during 1st trimester and this decrease becomes more accentuated as pregnancy advances [26]. The decrease in serum concentration is explained by haemodilution phenomenon. Indeed the intravascular mass of

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albumin has been found to be normal in pregnancy and the ratio of synthesis or catabolism is unaltered in normal pregnancy compared to controls [27]. In the table 1 and figure 2, the total bilirubin levels in experimental groups are found to decrease from the control non pregnant groups but within normal range $p < 0.05$. Total bilirubin concentration are decreased during in all three trimesters of pregnancy [26,28,29]. As albumin is the transport protein for bilirubin, there is decrease of bilirubin along with albumin and so we can assume haemodilution may be partially involve in the decrease of bilirubin. The AST levels as seen in table 2 and figure 3 does not show much change in all the trimesters as $P > 0.05$. This supports majority of published studies, serum ALT and AST activity levels do not change during pregnancy or remain within normal limits established in non pregnant women[30,31].But in case of ALT, there is gradual increase in 1st , 2nd and 3rd trimester when compared with control non pregnant group. It is insignificant $P > 0.05$ in the 1st and 2nd trimester but is significant in 3rd trimester, $P < 0.001$.This supports few studies, a slight increase in ALT and or AST has been found in third trimester [30,28,29]. An increase in ALT or AST levels during labour might be due to contractions of the uterine muscles[32]. In the present study GGTP level seems to decrease slightly in the 2nd and 3rd trimester but this decrease is within the normal range, $P> 0.05$. Serum GGTP activity has usually been considered to be normal during pregnancy[33,34,35]. When compared with control non pregnant group the ALP values of the experimental group (all three trimesters) have increased significantly from 1st trimester onwards. In case of 1st trimester it is significant $P < 0.05$ and in case of 2nd and 3rd trimester the increase in ALP values become highly significant, $P < 0.001$. This increase during pregnancy is not due to an increase in the hepatic isoenzyme but rather largely due to the production of the placental isoenzyme[36,37]. During the third trimester there is also an increase in the production of the bone isoenzyme as documented by an increase in its serum level up to six weeks post delivery[38,39].

Conclusion:

1) The results indicated a significant increase of ALT in pregnant women in third trimester when compared with those of the control group.
2) The levels of AST activity did not show much change in pregnant women when compared with those of control group.
3) The ALP activity indicated higher significantly in third and second trimester when compared with control group.
4) Serum albumin decreases in all the trimesters of pregnancy when compared with non pregnant women.
5) Total bilirubin also maintains a lower level in pregnant women when compared with non pregnant women.

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