# ASSESSMENT AND RELATIONSHIP OF ELEVATED SERUM ALANINE TRANSAMINASE LEVEL IN REFERENCE WITH OBESITY


## ABSTRACT

Imbalances between energy gained and exhausted results in abnormal fat accumulation in the tissues of human body and ultimately leads to obesity. Obese people vary not only in the quantity of extra fat that they store, but also in the local distribution of fat within the body. Proportions of obesity are increasing alarmingly in many portions of the world. Pakistan is ranked $9^{th}$ among 188 countries in carrying obese population, in terms of absolute numbers. Alanine Transaminase is commonly used to identify patients with liver disease. This study was designed to access the level of alanine transaminase in obese subject in the population of Peshawar city, Khyber Pakhtunkhwa, Pakistan. To carry out this survey 300 subjects was examined for body mass index. Blood samples from only obese patients were collected at RMI Hospital, Peshawar, from December 2018 to June 2019 after taking inform consent. Whole blood samples were collected by venipuncture technique in gel tube, after blood clotting and serum was extracted after centrifugation at 3000 rpm for 5 minutes to analyze serum Alanine Aminotransferase level. Out of 300 cases, 148 (49.3%) cases were separated in the categories of obese individuals depending on body mass index values according to WHO criteria. Female with increased body mass index were 71 (48.0%) and 77 (52.0 %) were males. About 35 (46%) male patients showed elevated Alanine Aminotransferase level and 24 (33.8%) females. In both male and female with increase body mass index showed elevated serum Alanine Aminotransferase. From this study, it was concluded that there is relatively increase in serum Alanine Aminotransferase level with increase in body mass index and this raised enzyme levels is more common in obese males than obese females. Further study in this area with large sample size will be fruitful to find strong correlation between these two.




Keywords: Obesity, Alanine Aminotransferase, serum, Alanine Transaminase

## 1. INTRODUCTION

Obesity is a state of excessive or atypical fat accumulation in adipose tissue, to the magnitude that healthiness may be compromised Excess weight over the normal can be in the form of overweight or more dangerously being obese[1]. Obesity is a condition of energy imbalance between intake and expenditure[2]. Imbalance between energy gained and exhausted results in abnormal fat accumulation in the tissues of human body and ultimately leads to obesity[3]. Obesity is generally stated by Body Mass Index (BMI) which is calculated by dividing the weight of an individual in Kilograms by his/her height given in meter square[4]. A person is considered as obese with body mass index greater than $30kg/m^2$[5].

Obese people vary not only in the quantity of extra fat that they store, but also in the local distribution of fat within the body[6]. When an organism encounters food excess, conserves the nutrients either as glycogen for short-term storage or as lipids for longer storage duration, which may result in a consequent state of obesity[7]. Dissatisfaction with one's body weight today is so common. This dissatisfaction extends to all of the developed world and even the traditional approbation of corpulence in the developing world is eroding. Early in life, children say that they would prefer as friends' children depicted with missing legs or eyes or with any disability, rather than obesity. As adults, obese individuals say that they themselves would prefer to be blind or deaf or have any disability, rather than that of their obesity[8]. Overweight denotes the presence of excess body weight. Obesity denotes the presence of excess body fat. All obese persons are overweight, but all overweight persons are not necessarily obese as excess body weight may arise from muscle, bone or body water content[9].

Human obesity is one of the oldest reported health disorders and can be traced as early as 25,000 years. It still remains relatively a rare condition for most of the human history[10]. The first example is derived from medieval Japan, from the 12th century, there was a woman money-lender who became exceedingly wealthy. Because she ate all kinds of rich foodstuffs, her body became fat and her flesh too abundant. She could not walk easily and when doing so she needed the help of her servant-girls. Even with that assistance, however, she perspired profusely, gasped for breath and suffered without let-up. Her obesity was viewed as the result of over-eating, an association that was recognized at the time[11].

Proportions of obesity are increasing alarmingly in many portions of the world. World Health Organization refers to obesity as a worldwide epidemic and stated that obesity is a long-lasting disease dominant in both developed and developing countries. According to WHO over 1 billion overweight people in the world 300000 are obese[12]. Each year, more than 2.5 million deaths are linked to obesity[13]. Developing countries are progressively susceptible to the worldwide epidemic of obesity, which affects all sectors of the population, including men, women and children[14]. This problem of excess weight is not only highly prevalent but has also increased more than three folds since the 1980s. It is now apparent that most countries of the world, even those which have just recently struggled with undernutrition (underweight) and some that continue to do so, are experiencing increases in obesity prevalence[15]. Western countries are facing an epidemic of obesity with increasing rates. The extreme threat is in Middle East, Pacific Islands, Southeast Asia, and China. Obesity in the United States has been increasingly cited as a major health issue in recent decades. The rates of obesity in the United States are among the highest in the world with 74.6% of Americans being overweight or obese[16]. About one in four adult Americans are obese, based on self-reported weight and more than one in three would be classified as obese, based on objectively measured weight. These rates have roughly tripled in the past 20 years[17]. Consequently, cases of severe obesity (BMI more than 35 $kg/m^2$) account for an increasingly large proportion of the obese population over time. One in seven Americans are now severely obese and the total severe obesity prevalence is 35% in the USA[18]. In England, one in five adults are obese and that rate has also tripled over the last 20 years[19].

According to a global disease burden research, Pakistan is ranked 9$^{th}$ among 188 countries in carrying obese population, in terms of absolute numbers[20]. Recent figures for Pakistan suggest that 40 percent of Pakistani women of reproductive age are either overweight or obese[21]. Data from the National Health Survey of Pakistan for 1990-1994 discovered frequency of obesity in 25–44-year-olds (9% males, 14% females) in rural areas and (22% males, 37% females) in urban areas. For 45–64-year-olds, prevalence was greater (11% males, 19% females) in rural areas and (23 % males, 40% females) in urban areas respectively[22].

Obesity and its associated comorbidities have become major health problems in the world[23]. The association between obesity and the development of major complications in acute pancreatitis, fatty liver diseases, vascular inflammation and coronary heart disease, chronic obstructive pulmonary disease, risk of cerebral ischemia and brain injury, atherosclerotic vascular disease, myocardial infarction, and cancers are strongly linked to chronic inflammation. Elevated serum concentrations of C-reactive protein, interleukin IL-6, IL-8 and tumour necrosis factor (TNF)-$\alpha$ was observed in obese individuals with elevated insulin resistance[24]. Obesity in humans is associated with low-level inflammation. Obesity may be viewed as a form of chronic inflammation[25]. The inflammatory response triggered by obesity involves a number of well- known components of the typical classical inflammatory response to pathogens. These components include (i) systemic increases in circulating inflammatory cytokines and adipokines and acute phase proteins, (ii) recruitment of leukocytes to inflamed tissues, (iii) activation of tissue leukocytes, and Mediators of Inflammation 3, (iv) generation of reparative tissue responses[26]. Severe obesity is associated with greater adverse consequences than mild obesity[27].

It can lead to a number of other health related problems like hypertension, hyperlipidaemia, type II diabetes, cardiovascular diseases and hepatic steatosis. Obesity is also linked with morbidity and decreased life expectancy[28]. Excess body fat is an established risk factor for numerous chronic diseases and premature death[29]. Childhood obesity is also associated with cardiovascular risk factors, carotid intima media thickness and left ventricular mass in early or mid-adulthood[30].

Epidemiologic evidence suggests that obesity increases the risk for cirrhosis. In autopsy series, obesity was identified as the only risk factor for liver disease in 12% of cirrhotic subjects. Conversely, cirrhosis is approximately six times more prevalent in obese individuals than in the general population[31]. The pathogenesis of liver disease associated with obesity is unknown. Gradual progression from hepatic steatosis to steatohepatitis and eventually, to cirrhosis is thought to occur. Hepatic steatosis is common in obese individuals and has been documented in individuals who are as little as 10% above ideal body weight. Although at least 40% of morbidly obese patients who undergo abdominal surgery to treat obesity exhibit hepatic steatosis, only a fraction of obese individuals with steatosis develops cirrhosis[32]. Fewer than 10% of obese subjects with steatohepatitis exhibit normal liver morphology and almost 40% have become cirrhotic[33]. Fatty liver is most prevalently associated with obesity and diabetes through unknown mechanisms. It is not clear whether the effects of obesity on the liver can be separated from those of impaired glucose tolerance (IGT) or NIDDM or whether they have any relationship to the pathogenesis of inflammation in Non-alcoholic steatohepatitis[34]. Non-alcoholic fatty liver disease (NAFLD) is also common in severely obese subjects and can progress to cirrhosis and liver failure[35].

The patients with high liver fat content were more obese than those with a low liver fat content. Septal fibrosis occurs frequently in obese patients with abnormal liver function tests[36]. A bright liver at ultrasounds and increased levels of hepatic enzymes are also common in obesity. High levels of alanine and aspartate transaminase (ALT and AST), as well as raised gamma-glutamyl transpeptidase (GGT) activity are frequently observed in association with body mass index (BMI) and raised insulin levels[37].

Alanine aminotransferase (ALT) is a biological catalyst. ALT is also called as serum glutamic pyruvic transaminase (GPT)[38]. The serum aminotransferases discovered in 1927 by Needham[39]. Serum ALT level is commonly used as an early marker for assessment of various liver diseases[40]. ALT has been used to identify patients with liver disease for almost 50 years. For detection of liver diseases, ALT is thought to be a more specific indicator than aspartate aminotransferase, an enzyme found in cytosol and mitochondria[41]. Serum levels of ALT normally are low, 167 to 667 nkat/L (10 to 40 U/L) in most laboratories however, normal values may vary greatly among laboratories. The absolute height of the ALT elevation does not correlate with the extent of liver cell damage[42].

However, when body tissue or an organ such as the liver is diseased or damaged, additional ALT is released into the bloodstream, causing levels of the enzyme to rise. Therefore, the amount of ALT in the blood is directly related to the extent of the tissue damage. After severe damage, ALT can reach higher levels (up to 50 times greater than normal). On the other hand, the concentration of ALT sometimes can help determine whether the liver or another organ has been damaged[43].

Monitoring of trends in the prevalence of overweight and obesity depends on household surveys. Many health interview surveys include questions on self-reported weight and height that have been used to monitor trends overtime. Examination surveys provide direct measurements of weight and height but many fewer countries conduct repeated national examination surveys and estimates from them may be biased because of low participation rates[44]. Mean BMI estimates have been used to predict levels of overweight and obesity over the period 1980-2008[45].

Developed imaging methods, such as computed tomography (CT) and magnetic resonance imaging (MRI), can provide the most precise estimate of the location and amount of adipose tissue in various body regions. The use of these methods in epidemiologic studies is limited because of high cost, complexity of operation and lack of portability of equipment. In comparison with these methods, dual-energy x-ray absorptiometry (DXA) provides a more practical approach to directly measuring body fatness[46]. These are also expansive techniques and are limited to high research surveys[47].

Anthropometric indices, such as body mass index (BMI) and waist circumference, remain the most commonly used measurements of adiposity in epidemiologic studies because of their simplicity[48]. Skinfold thickness also is utilized to estimate body fatness and a number of prediction equations incorporating different skinfold measurements have been developed[49].

Body mass index (BMI) is a measure of weight adjusted for height. Weight and height are relatively easy to measure and because BMI is relatively highly correlated with body fat. BMI is often used in epidemiologic studies to assess adiposity[50]. It requires minimal training and repeat values can be obtained with good precision[51]. There is strong correlations between BMI and DXA measurements of adiposity in various populations[52]. A variety of other, more elaborate, methods are also used as measures of body weight including underwater weighing, estimates of total body water, total body electrical conductivity, total body potassium, magnetic resonance imaging and computed tomography[53].

Obese individuals are not a homogeneous group, but highly heterogeneous. Obese individual differs in terms of health status and functional ability[24]. Two distinct subtypes of obesity have been proposed. One type is “metabolically healthy obese” (MHO) and the other is “metabolically unhealthy” obese (MUHO). It is interesting to note that approximately 20–30% of the adult obese population remains at the level of relatively “metabolically healthy” obesity (MHO) as compared to those with “metabolically unhealthy” obesity[54]. Individuals in the first subtype have also been termed “metabolically normal obese”, “metabolically healthy but obese”, “obese metabolically normal”, “metabolically benign obesity” or uncomplicated obesity[55]. Significantly, both subtypes associate with different inflammatory profiles. MUHO exhibit increased levels of inflammation compared to other normal-weight individuals[56], while MHO exhibit reduced levels of inflammation compared to other obese individuals[57]. MHO individuals demonstrate an absence of impaired glucose tolerance, dyslipidaemia, hyperuricemia, and hypertension[58].

Obesity shows both genetic and environmental relations, suggesting individual susceptibility that relates with adverse environmental situations. Although the particular bases of obesity remain to be entirely elucidated, relations with demographic, socio-cultural, biological, behavioural and lifestyle factors have been observed[59]. Decreased physical activity and overconsumption of cheap, energy-dense food have also led to globally increasing incidence of obesity with tripled rates in the last 20 years[22].

The increased prevalence of excess weight is not the only concern for nations, rather inequalities in its prevalence among population groups is another concern. Research across the countries shows that various economic and noneconomic factors are responsible for having more body weight than normal[60]. Eating fast food and snacks were significantly more likely to be overweight. Physical activity more than twice a week showed a significant inverse association with overweight and obesity. The risk of being overweight and obese significantly increased with the involvement in sedentary lifestyle included television viewing, working on computer and playing video games. Skipping breakfast also showed a significant independent positive association with BMI[61]. Household income has an inverse relation with body mass index. There is negative relationship between education and the probability of being obese. Obesity risk was greater in men and women with fewer years of education, lower occupational status and poorer economic circumstances. Contrary to women, poorer men are less likely to be obese[62].

Studies show that there is joint and separate effect of smoking and alcohol usage to elevated levels of ALT in men[63]. Keeping an eye on these studies, this study was designed to correlate the elevated ALT levels with obese subject both male and female. Study includes the District Haripur and no reported data is available linked to our study in Haripur, Khyber Pakhtunkhwa, Pakistan.

### Aims and Objectives of study

- To screen the patients for Alanine Aminotransferase and obesity.
- To find relationship between obesity and elevated Alanine Aminotransferase level.

## 2. METHODS AND MATERIALS

### 2.1 Place of study

This study was carried out in RMI Hospital, Peshawar city after taking consent forms from patients.

### 2.2 Inclusion criteria

Patients visited RMI Hospital, Peshawar during December 2018 to June 2019 and reported body mass index >30kg/m$^2$ were be enrolled in this study.

### 2.3 Exclusion criteria

All those patients reported ideal body weight, body mass index $< 25 kg/m^2$, obese but have Hepatitis B, Hepatitis C and jaundice history and those not willing to take part in this study were excluded.

### 2.4 Sample Collection

About 5 mL of clotted blood sample was collected from the patient in plain tube aseptically by venipuncture technique after taking inform consent. For separation of serum from clotted blood, it was centrifuge at 3000 rpm for 5 minutes and was store at -20$^oC$ before further analysis. Screening test was done on Automatic Bio Cor Chemistry Analyzer of Merck Company of Germany.

### 2.5 Calculation of Body Mass Index

Body weight was measured in light clothing and without shoes to the nearest half-kg. Height was measured to the nearest half centimeter[64]. The following formulae was applied during the data collection process for the determination of obesity[5].

$$\text{Body Mass Index (BMI)} = \text{Weight (Kg)} / \text{Height } (m^2)$$

### 2.6 Analysis of ALT Enzyme

Non hemolyzed Serum was analyzed for ALT level by standard assay ALT/GPT Spectrum kit (Catalog No. 264 001) using automatic Bio Cor Chemistry Analyzer. ALT/GPT spectrum kit contains reagents that are Reagent 1 and Reagent 2. Reagent 1 (R1 Buffer) contains Phosphate Buffer (100 mmol/L), DL Alanine (200 mmol/L), 2- Oxoglutarate (6 mmol/L) and sodium azido (12 mmol/L). Reagent 2 (R2) contains 2, 4 dinitrophenylhydrazine (2.0 mmol/L). Working Solution was prepared by mixing R1 buffer and R2 solution in ratio of 5:1. Working solution was stable for 7 days at 4 °C.

0.5 mL of working reagent was aspirated in the sterile test tube and 100 µL of sample was added to working solution in proportion of 1:60. The mixture was incubated for 30 minutes at 37 °C. Absorbance of the sample was measured at 546 nm after 5 minutes. Positive and Negative Control samples available in these commercially available kits was used to check to ensure the quality control of the procedure and kits.

### 2.7 Statistical Analysis

Data collected was analyzed by suitable statistical tools.

## RESULTS and DISCUSSION

The current research was conducted in RMI Hospital, Peshawar KPK Pakistan during December 2018 to June 2019. A total of 300 people who seems to be obese (159 females and 141 males) was examined during the entire period for obesity using BMI. Out of these, 148 (49.3%) cases are obese having BMI greater than 29.9 kg/m$^2$, while 152 (50.7%) cases are in over weight range (BMI in between 25.0 to 29.9 kg/m$^2$). About of 148 obese cases, 71 (48.0%) cases are female and 77 (52.0 %) are males as shown in table 1 and figure 1.

Alt level was assessed in the serum of all obese people. About 35 (46%) male patients show elevated ALT and 42 (54%) are in normal range as shown in table 2 and figure 2. In females about, 23 (32.4%) cases are ALT elevated while 48 (77.6%) are normal as shown in table 3 and figure 3.

BMI wise distribution shows the increase in percentage of ALT elevated cases. In males 19 out of 59 cases had elevated ALT having BMI in range of 30.0 to 35.0 kg/m$^2$ and 40 cases falls in normal range of ALT. Cases with 35.1 to 40.0 kg/m$^2$ BMI had 7 out of 13 cases with elevated ALT and BMI above 40 Kg/m$^2$ had 4 out of 5 cases in ALT elevated range as shown in table figure 4.

**Table 1:** Male and female population of Obese individuals

| Parameter | Total No. of Obesity cases | Total No. of Overweight cases | Total no. of cases |
|---|---|---|---|
| **Over All** | 148 | 152 | 300 |
| **Males** | 77 | 82 | 159 |
| **Females** | 71 | 70 | 141 |

In females 16 out of 52 cases had elevated ALT having BMI in range of 30.0 to 35.0 kg/m$^2$ and 36 cases falls in normal range of ALT. cases with BMI 35.1 to 40.0 kg/m$^2$ had 5 out of 14 cases with elevated ALT and BMI above 40 Kg/m$^2$ had 2 out of 4 cases in ALT elevated range.


**Figure 1:** Male and female population of Obese individuals

**Table 2:** Frequency of ALT Level in Obese Male

| Parameter | Elevated | Normal | Total |
|---|---|---|---|
| **Male** | 35 (46%) | 42 (54%) | 77 |

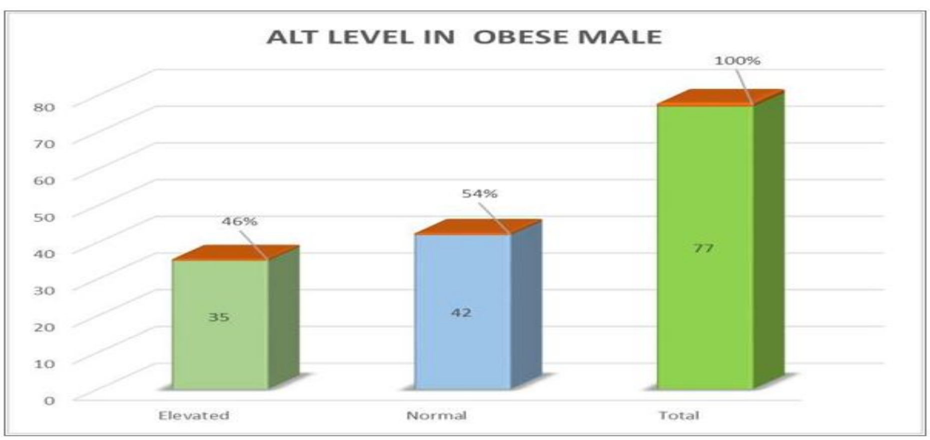


Fig. 2: Frequency of ALT Level in Obese Male

**Table 3:** Frequency of ALT level in Obese Female

| Parameter | Elevated | Normal | Total |
|---|---|---|---|
| **Female** | 24 (33.8%) | 48 (66.2%) | 71 |

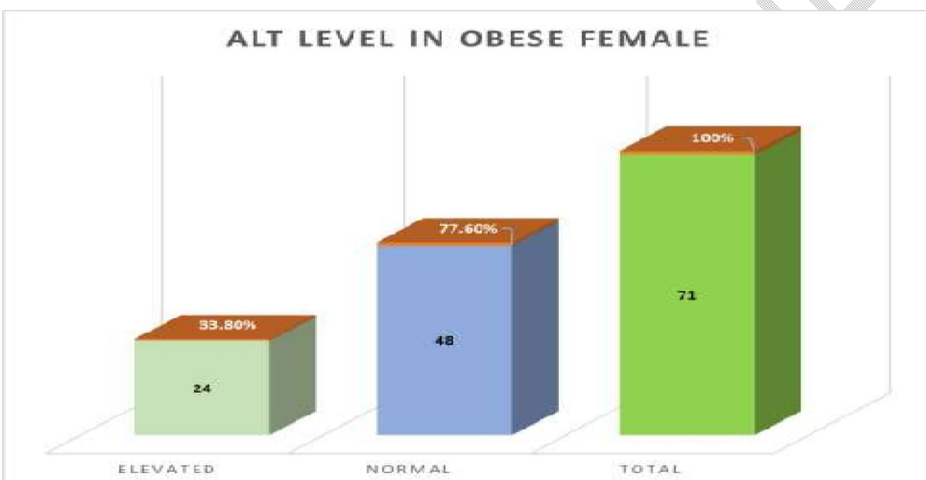


**Figure 3:** Frequency of ALT Level in Obese Female

**Table 4:** Frequency of ALT Level in Obese Male (BMI Wise Distribution)

| Parameter | 30.0 to 35.0 kg/m$^2$ | 35.1 to 40.0 kg/m$^2$ | Above 40.0 kg/m$^2$ |
|---|---|---|---|
| **Elevated ALT** | 19 (32.2%) | 7 (53.8%) | 4 (80.0%) |
| **Normal ALT** | 40 (67.8%) | 6 (46.2%) | 1 (20.0%) |

**Figure 4:** Frequency of ALT level in Obese Male (BMI Wise Distribution)

**Table 5:** Frequency of ALT level in Obese Female (BMI Wise Distribution)

| Parameter | 30.0 to 35.0 kg/m$^2$ | 35.1 to 40.0 kg/m$^2$ | Above 40.0 kg/m$^2$ |
|---|---|---|---|
| **Elevated ALT** | 16 (30.7%) | 5 (35.7%) | 2 (50.0%) |
| **Normal ALT** | 36 (69.2%) | 9 (64.3%) | 2 (50.0%) |

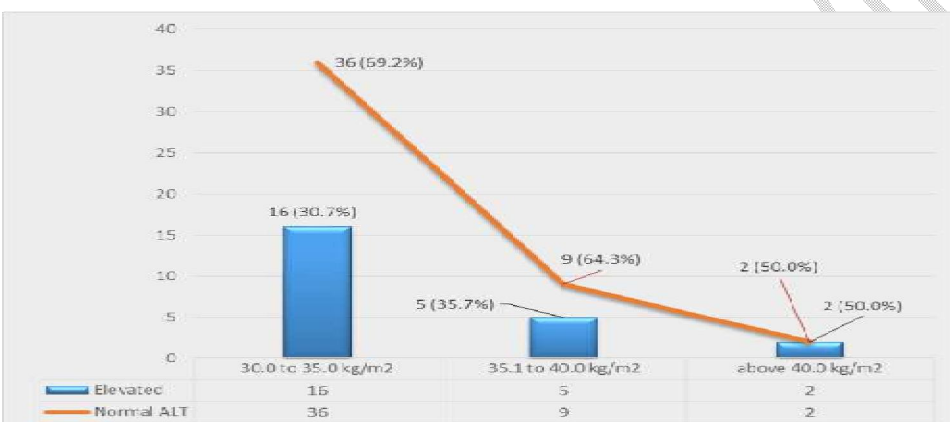


**Figure 5:** Frequency of ALT level in Obese Female (BMI Wise Distribution

## DISCUSSION

Obesity is a condition of energy imbalance between intake and expenditure[7]. Human obesity is one of the oldest reported health disorders and can be traced as early as 25,000 years[10]. Each year, more than 2.5 million deaths are linked to obesity[13]. Pakistan is ranked 9$^{th}$ among 188 countries in carrying obese population[65]. Data from the National Health Survey of Pakistan for 1990-1994 discovered frequency of obesity in 25–44-year-old (9 % males, 14 % females) in rural areas and (22 % males, 37 % females) in urban areas. For 45–64-year-olds, prevalence was greater (11 % males, 19 % females) in rural areas and (23 % males, 40 % females) in urban areas respectively.

In the present study, a total of 300 fat peoples were observed, out of which 148 (49.30%) are obese (77 males and 71 females). ALT level was assessed in the serum of all obese people. Elevation of ALT level in serum were found more in male cases than female. About 46.0% of total male patients show elevated Alt and 32.4% of total female cases are ALT elevated. BMI distribution shows that elevated ALT level is more in patients with BMI more than 40.0 kg/m$^2$, followed by low percentage is found in cases having BMI ranges to 35.1 kg/m$^2$ to 40.0 kg/m$^2$ and least number of cases with elevated serum ALT are reported with 30.0 kg/m$^2$ to 35.0 Kg/m$^2$ BMI. Percentage of

ALT elevated cases are more in more obese patients.

In contrast to our study,[66] reported that in both sex, the percentage increase in the geometric mean of liver enzyme activity of the obese subjects (BMI greater than 30 kg/m$^2$) compared with that of the normal subjects (BMI less than or equal to 25 kg/m$^2$). Our observations demonstrate a similar relation between BMI and ALT activity. Another study by [63], found that three liver enzymes (ALT, AST and GGT) showed marked increases in mean levels with increasing body mass index (BMI). The prevalence of increased ALT values in obese subjects (BMI greater than or equal to 31 kg/m2) was more than eight times that in those with normal weight (BMI less than or equal to 25 kg/m2), even after allowing for the confounding effect of alcohol consumption. They also mentioned that levels of liver enzymes (ALT, AST and GGT) were positively correlated with levels of alcohol consumption. Decreasing levels of physical activity were also associated with increases in mean ALT and GGT levels. Cigarette smoking showed only a weak effect on ALT and AST. No other such study was found related to obesity and ALT or liver enzymes.

Study by[67], found that with the rise in ALT, a significant decrease in insulin sensitivity. Fasting proinsulin and insulin rose significantly with rising ALT and GGT elevations. They also reported that 48 % of subjects with fatty liver had abnormal ALT levels. ALT with relation to alcohol consumption was studied by [63] and reported that there were no changes of clinical significance in AST or ALT activities with smoking while show positive correlation with alcohol consumption. In a univariate analysis by[68], men with ALT in the top quartile (ALT >29 units/l) had an elevated risk for diabetes versus those in the bottom quartile (<17 units/l).

Relation between liver enzymes, CRP and bilirubin with metabolic syndrome and other measurement was studied by [69]found that subjects with metabolic syndrome had significantly elevated concentrations of AST, ALT and ALK. Those who developed metabolic syndrome also had higher concentrations of ALT and ALK and lower levels of the AST-to-ALT ratio. ALT, ALK, bilirubin and CRP showed positive correlations and the AST to- ALT ratio inverse correlations, with anthropometric measures, fasting glucose, insulin, and triglyceride and inverse correlations with HDL. The magnitude of these associations was strongest for ALT, especially those for waist circumference, fasting glucose and fasting insulin and ALK were inversely correlated with alcohol consumption. The results were similar in ex alcohol consumers. After adjustment for age, sex, clinical center, ethnicity, and alcohol intake, the natural logs of ALT, the AST-to- ALT ratio, ALK and CRP were significantly associated with 5-year risk of incident metabolic syndrome.

## References


[1] M. Pigeyre, M. Saqlain, M. Turcotte, G. K. Raja, and D. Meyre, "Obesity genetics: insights from the Pakistani population," *Obes. Rev.*, vol. 19, no. 3, pp. 364–380, 2018, doi: 10.1111/obr.12644.

[2] Y. T. Tung, H. L. Chen, H. S. Wu, M. H. Ho, K. Y. Chong, and C. M. Chen, "Kefir Peptides Prevent Hyperlipidemia and Obesity in High-Fat-Diet-Induced Obese Rats via Lipid Metabolism Modulation," *Mol. Nutr. Food Res.*, vol. 62, no. 3, pp. 1–9, 2018, doi: 10.1002/mnfr.201700505.

[3] Y. Fan and O. Pedersen, "Gut microbiota in human metabolic health and disease," *Nat. Rev. Microbiol.*, vol. 19, no. 1, pp. 55–71, 2021, doi: 10.1038/s41579-020-0433-9.

[4] G. Ojo and O. Adetola, "The Relationship between Skinfold Thickness and Body Mass Index in Estimating Body Fat Percentage on Bowen University Students," vol. 3, no. 3, 2017.

[5] A. Sadia, E. Strodl, N. G. Khawaja, R. Kausar, and M. J. Cooper, "Understanding eating and drinking behaviours in Pakistani university students : A conceptual model through qualitative enquiry," *Appetite*, vol. 161, no. January, p. 105133, 2021, doi: 10.1016/j.appet.2021.105133.

[6] A. Imtiaz, S. Afaq, and M. N. Khan, "Prevalence and patterns of physical activity among school aged adolescents in Pakistan : a systematic review and meta-analysis," *Int. J. Adolesc. Youth*, vol. 25, no. 1, pp. 1036–1057, 2020, doi: 10.1080/02673843.2020.1831559.

[7] V. E. Attari, A. Malek, M. Zeinab, S. Mahluji, S. Zununi, and V. Alireza, "A systematic review of the anti  obesity and weight lowering effect of ginger ( Zingiber officinale Roscoe ) and its mechanisms of action," no. July, pp. 1–9, 2017, doi: 10.1002/ptr.5986.

[8] S. R. Karasu, "Of Mind and Matter : Psychological Dimensions in Obesity," vol. 66, no. 2, pp. 111–128, 2012.

[9] A. A. Herekar *et al.*, "Primary headache disorders in the adult general population of Pakistan – a cross sectional nationwide prevalence survey," pp. 1–9, 2017, doi: 10.1186/s10194-017-0734-1.

[10] R. J. Johnson, P. Andrews, and M. A. Lanaspa, "Perspective : A Historical and Scienti fi c Perspective of Sugar and Its Relation with Obesity and Diabetes 1 – 4," 2017, doi: 10.3945/an.116.014654.412.

[11] R. Kielmann and S. Herpertz, "Psychosocial aspects of obesity surgery," *Int. J. Obes.*, vol. 26, no. Supplement 1, pp. S74–S74, 2002.

[12] A. El-Shaheed, N. Mahfouz, M. Elabd, and S. Sallam, "Physical activity patterns in Egyptian obese and nonobese adolescents assessed using a validated WHO questionnaire," *J. Arab Soc. Med. Res.*, vol. 15, no. 1, p. 6, 2020, doi: 10.4103/jasmr.jasmr_27_19.

[13] S. H. Raza, W. Haq, and M. Sajjad, "COVID-19: A Psychosocial Perspective," *Front. Psychol.*,

vol. 11, no. November 2019, pp. 1–12, 2020, doi: 10.3389/fpsyg.2020.554624.

[14] T. Darebo, A. Mesfin, and S. Gebremedhin, “Prevalence and factors associated with overweight and obesity among adults in Hawassa city, southern Ethiopia: A community based cross-sectional study,” *BMC Obes.*, vol. 6, no. 1, pp. 1–10, 2019, doi: 10.1186/s40608-019-0227-7.

[15] D. Petrakis *et al.*, “Obesity □ a risk factor for increased COVID□19 prevalence, severity and lethality (Review),” *Mol. Med. Rep.*, vol. 22, no. 1, pp. 9–19, 2020, doi: 10.3892/mmr.2020.11127.

[16] S. Azulai *et al.*, “Sleeve gastrectomy is associated with a greater reduction in plasma liver enzymes than bypass surgeries—a registry-based two-year follow-up analysis,” *J. Clin. Med.*, vol. 10, no. 5, pp. 1–10, 2021, doi: 10.3390/jcm10051144.

[17] J. F. Masa, J. L. Pépin, J. C. Borel, B. Mokhlesi, P. B. Murphy, and M. Á. Sánchez-Quiroga, “Obesity hypoventilation syndrome,” *Eur. Respir. Rev.*, vol. 28, no. 151, pp. 1–14, 2019, doi: 10.1183/16000617.0097-2018.

[18] M. Claudy, G. Doyle, L. Marriott, N. Campbell, and G. O’Malley, “Are Sugar-Sweetened Beverage Taxes Effective? Reviewing the Evidence Through a Marketing Systems Lens,” *J. Public Policy Mark.*, vol. 40, no. 3, pp. 403–418, 2021, doi: 10.1177/0743915620965153.

[19] D. P. Schauer *et al.*, “Bariatric Surgery and the Risk of Cancer in a Large Multisite Cohort,” *Ann. Surg.*, vol. 269, no. 1, pp. 95–101, 2019, doi: 10.1097/SLA.0000000000002525.

[20] S. A. Ali *et al.*, “Prevalence and determinants of anemia among women of reproductive age in Thatta Pakistan: Findings from a cross-sectional study,” *PLoS One*, vol. 15, no. 9 September, pp. 1–16, 2020, doi: 10.1371/journal.pone.0239320.

[21] A. P. Hills *et al.*, “Epidemiology and determinants of type 2 diabetes in south Asia,” *Lancet Diabetes Endocrinol.*, vol. 6, no. 12, pp. 966–978, 2018, doi: 10.1016/S2213-8587(18)30204-3.

[22] N. A. Althumiri *et al.*, “Obesity in Saudi Arabia in 2020: Prevalence, distribution, and its current association with various health conditions,” *Healthc.*, vol. 9, no. 3, pp. 1–8, 2021, doi: 10.3390/healthcare9030311.

[23] B. Han, X. H. He, Y. Q. Liu, G. He, C. Peng, and J. L. Li, “Asymmetric organocatalysis: An enabling technology for medicinal chemistry,” *Chem. Soc. Rev.*, vol. 50, no. 3, pp. 1522–1586, 2021, doi: 10.1039/d0cs00196a.

[24] O. Bosello and A. Vanzo, “Obesity paradox and aging,” *Eat. Weight Disord.*, vol. 26, no. 1, pp. 27–35, 2021, doi: 10.1007/s40519-019-00815-4.

[25] D. Chen *et al.*, “Osteoarthritis: Toward a comprehensive understanding of pathological

mechanism," *Bone Res.*, vol. 5, no. September 2016, 2017, doi: 10.1038/boneres.2016.44.

[26] G. Hajishengallis and T. Chavakis, "Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities," *Nat. Rev. Immunol.*, vol. 21, no. 7, pp. 426–440, 2021, doi: 10.1038/s41577-020-00488-6.

[27] A. Pearce, S. Hope, L. Griffiths, M. Cortina-Borja, C. Chittleborough, and C. Law, "What if all children achieved WHO recommendations on physical activity? Estimating the impact on socioeconomic inequalities in childhood overweight in the UK Millennium Cohort Study," *Int. J. Epidemiol.*, vol. 48, no. 1, pp. 134–147, 2019, doi: 10.1093/ije/dyy267.

[28] M. Amjad, M. I. Zafar, A. A. Maan, and M. T. Kashif, "Causes and Implications of Obesity in Urban School Going Children," vol. 12, no. 5, pp. 202–207, 2018.

[29] A. De Lorenzo, S. Gratteri, P. Gualtieri, A. Cammarano, P. Bertucci, and L. Di Renzo, "Why primary obesity is a disease?," *J. Transl. Med.*, vol. 17, no. 1, pp. 1–13, 2019, doi: 10.1186/s12967-019-1919-y.

[30] B. Rocca *et al.*, "Antithrombotic therapy and body mass: An expert position paper of the ESC Working Group on Thrombosis," *Eur. Heart J.*, vol. 39, no. 19, pp. 1672–1686, 2018, doi: 10.1093/eurheartj/ehy066.

[31] S. A. Polyzos, J. Kountouras, and C. S. Mantzoros, "Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics," *Metabolism.*, vol. 92, pp. 82–97, 2019, doi: 10.1016/j.metabol.2018.11.014.

[32] A. J. Sanyal, "Past, present and future perspectives in nonalcoholic fatty liver disease," *Nat. Rev. Gastroenterol. Hepatol.*, vol. 16, no. 6, pp. 377–386, 2019, doi: 10.1038/s41575-019-0144-8.

[33] M. Heikenwalder, "From NASH to HCC: current concepts and future challenges," *Nat. Rev. Gastroenterol. Hepatol.*, doi: 10.1038/s41575-019-0145-7.

[34] A. Lonardo, S. Leoni, K. A. Alswat, and Y. Fouad, "History of Nonalcoholic Fatty Liver Disease," pp. 1–38.

[35] F. W. B. Sanders and J. L. Griffin, "De novo lipogenesis in the liver in health and disease : more than just a shunting yard for glucose," vol. 91, pp. 452–468, 2016, doi: 10.1111/brv.12178.

[36] Y. Kamada, K. Morishita, M. Koseki, M. Nishida, and T. Asuka, "Serum Mac-2 Binding Protein Levels Associate with Metabolic Parameters and Predict Liver Fibrosis Progression in Subjects with Fatty Liver Disease : A 7-Year Longitudinal Study", doi: 10.3390/nu12061770.

[37] A. Mosca, N. Panera, A. Crudele, and A. Alisi, "Non-invasive diagnostic tools for pediatric NAFLD: where are we now?," *Expert Rev. Gastroenterol. Hepatol.*, vol. 0, no. 0, 2020, doi:

10.1080/17474124.2020.1801413.

[38] A. N. F. Neamat-allah, E. A. Mahmoud, Y. Abd, and E. Hakim, "Fish and Shellfish Immunology Efficacy of dietary Nano-selenium on growth , immune response , antioxidant , transcriptomic profile and resistance of Nile tilapia , Oreochromis niloticus against Streptococcus iniae infection," *Fish Shellfish Immunol.*, vol. 94, no. August, pp. 280–287, 2019, doi: 10.1016/j.fsi.2019.09.019.

[39] N. Aggarwal, A. Singh, A. Agarwal, V. Dwarakanathan, A. K. Verma, and R. Amarchand, "Prevalence of elevated alanine aminotransferase levels in adult participants from a community-based study from northern part of India," 2020.

[40] R. Ghamrawi and M. Gunaratne, "COVID-19 and Sex Differences," *Mayo Clin. Proc.*, vol. 95, no. 10, pp. 2189–2203, 2020, doi: 10.1016/j.mayocp.2020.07.024.

[41] P. Y. Kwo, S. M. Cohen, and J. K. Lim, "ACG Clinical Guideline : Evaluation of Abnormal Liver Chemistries," no. September, pp. 1–18, 2016, doi: 10.1038/ajg.2016.517.

[42] G. Pathophysiology, "World Journal of," vol. 5330, no. 2, 2017.

[43] A. N. F. Neamat-allah, Y. Abd, E. Hakim, and E. A. Mahmoud, "Alleviating effects of β -glucan in Oreochromis niloticus on growth performance , immune reactions , antioxidant , transcriptomics disorders and resistance to Aeromonas sobria caused by atrazine," no. November 2019, pp. 1–12, 2020, doi: 10.1111/are.14529.

[44] D. Glovaci, W. Fan, and N. D. Wong, "S11886-019-1107-Y.Pdf," *Curr. Cardiol. Rep.*, vol. 21, no. 21, pp. 1–8, 2019, [Online]. Available: https://doi.org/10.1007/s11886-019-1107-y

[45] L. M. Jaacks *et al.*, "The obesity transition: stages of the global epidemic," *Lancet Diabetes Endocrinol.*, vol. 7, no. 3, pp. 231–240, 2019, doi: 10.1016/S2213-8587(19)30026-9.

[46] K. M. Gadde, C. K. Martin, H. R. Berthoud, and S. B. Heymsfield, "Obesity: Pathophysiology and Management," *J. Am. Coll. Cardiol.*, vol. 71, no. 1, pp. 69–84, 2018, doi: 10.1016/j.jacc.2017.11.011.

[47] M. O. Fosbøl and B. Zerahn, "Contemporary methods of body composition measurement," *Clin. Physiol. Funct. Imaging*, vol. 35, no. 2, pp. 81–97, 2015, doi: 10.1111/cpf.12152.

[48] M. Borga *et al.*, "Advanced body composition assessment: From body mass index to body composition profiling," *J. Investig. Med.*, vol. 66, no. 5, pp. 887–895, 2018, doi: 10.1136/jim-2018-000722.

[49] J. Bovet, "Evolutionary theories and men's preferences for women's waist-to-hip ratio: Which hypotheses remain? A systematic review," *Front. Psychol.*, vol. 10, no. JUN, pp. 1–20, 2019, doi:

10.3389/fpsyg.2019.01221.

[50] V. L. Roger *et al.*, “Heart disease and stroke statistics-2011 update: A report from the American Heart Association,” *Circulation*, vol. 123, no. 4, pp. 18–209, 2011, doi: 10.1161/CIR.0b013e3182009701.

[51] T. Brown *et al.*, “Interventions for preventing obesity in children,” *Cochrane Database Syst. Rev.*, vol. 2019, no. 7, 2019, doi: 10.1002/14651858.CD001871.pub4.

[52] I. Romieu *et al.*, “Energy balance and obesity: what are the main drivers?,” *Cancer Causes Control*, vol. 28, no. 3, pp. 247–258, 2017, doi: 10.1007/s10552-017-0869-z.

[53] Y. Park, L. L. Peterson, and G. A. Colditz, “The plausibility of obesity paradox in cancer,” *Cancer Res.*, vol. 78, no. 8, pp. 1898–1903, 2018, doi: 10.1158/0008-5472.CAN-17-3043.

[54] M. E. Piché, A. Tchernof, and J. P. Després, “Obesity Phenotypes, Diabetes, and Cardiovascular Diseases,” *Circ. Res.*, pp. 1477–1500, 2020, doi: 10.1161/CIRCRESAHA.120.316101.

[55] M. González, “Alerta por aumento de la violencia contra los niños durante la emergencia.,” *Primicias*, vol. 9, pp. 1–8, 2020.

[56] S. Gómez-Zorita, M. Queralt, M. A. Vicente, M. González, and M. P. Portillo, “Metabolically healthy obesity and metabolically obese normal weight: a review,” *J. Physiol. Biochem.*, vol. 77, no. 1, pp. 175–189, 2021, doi: 10.1007/s13105-020-00781-x.

[57] A. Chait and L. J. den Hartigh, “Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease,” *Front. Cardiovasc. Med.*, vol. 7, no. February, pp. 1–41, 2020, doi: 10.3389/fcvm.2020.00022.

[58] M. Longo *et al.*, “Adipose tissue dysfunction as determinant of obesity-associated metabolic complications,” *Int. J. Mol. Sci.*, vol. 20, no. 9, 2019, doi: 10.3390/ijms20092358.

[59] C. Stryjecki, A. Alyass, and D. Meyre, “Ethnic and population differences in the genetic predisposition to human obesity,” *Obes. Rev.*, vol. 19, no. 1, pp. 62–80, 2018, doi: 10.1111/obr.12604.

[60] Y. Wang, L. Zhao, L. Gao, A. Pan, and H. Xue, “Health policy and public health implications of obesity in China,” *Lancet Diabetes Endocrinol.*, vol. 9, no. 7, pp. 446–461, 2021, doi: 10.1016/S2213-8587(21)00118-2.

[61] R. E. Rhodes *et al.*, “Development of a consensus statement on the role of the family in the physical activity, sedentary, and sleep behaviours of children and youth,” *Int. J. Behav. Nutr. Phys. Act.*, vol. 17, no. 1, pp. 1–31, 2020, doi: 10.1186/s12966-020-00973-0.

[62] L. Wang *et al.*, “Body-mass index and obesity in urban and rural China: findings from

consecutive nationally representative surveys during 2004–18," *Lancet*, vol. 398, no. 10294, pp. 53–63, 2021, doi: 10.1016/S0140-6736(21)00798-4.

[63] S. M. Rutledge and A. Asgharpour, "Smoking and liver disease," *Gastroenterol. Hepatol.*, vol. 16, no. 12, pp. 617–625, 2020.

[64] C. Wu, W. Yeh, W. Hsu, M. Islam, and Y. J. Li, "Computer Methods and Programs in Biomedicine," *Comput. Methods Programs Biomed.*, vol. 170, pp. 23–29, 2019, doi: 10.1016/j.cmpb.2018.12.032.

[65] M. Dehlin, L. Jacobsson, and E. Roddy, "Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors," *Nat. Rev. Rheumatol.*, vol. 16, no. 7, pp. 380–390, 2020, doi: 10.1038/s41584-020-0441-1.

[66] M. E. Abd El-Hack *et al.*, "Impacts of supplementing broiler diets with biological curcumin, zinc nanoparticles and bacillus licheniformis on growth, carcass traits, blood indices, meat quality and cecal microbial load," *Animals*, vol. 11, no. 7, 2021, doi: 10.3390/ani11071878.

[67] Z. W. Bitew, A. Alemu, E. G. Ayele, Z. Tenaw, A. Alebel, and T. Worku, "Metabolic syndrome among children and adolescents in low and middle income countries: a systematic review and meta-analysis," *Diabetol. Metab. Syndr.*, vol. 12, no. 1, 2020, doi: 10.1186/s13098-020-00601-8.

[68] R. Kuroda, K. Nogawa, Y. Watanabe, H. Morimoto, K. Sakata, and Y. Suwazono, "Association between high-sensitive c-reactive protein and the development of liver damage in japanese male workers," *Int. J. Environ. Res. Public Health*, vol. 18, no. 6, pp. 1–11, 2021, doi: 10.3390/ijerph18062985.

[69] E. J. Benjamin *et al.*, *Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association*, vol. 139, no. 10. 2019. doi: 10.1161/CIR.0000000000000659.