

Investigation of Association Between Severity of Diabetes and Alkaline Phosphatase in Human

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Abstract: Liver enzymes are commonly used in the evaluation of patients with a range of diseases. The present study was conducted to find an association between the abnormal serum glucose level (SGL) and alkaline phosphatase (ALP) activity. 165 patients (male, female and Shemale) of various diseases were involved in this study. Blood samples of these patients were centrifuged and serum was used for estimation of glucose and ALP by selectra E method. Result showed that 80% patients were from urban area and 20% from rural areas of district Gujranwala, Pakistan. 10.3% of total patients showed normal/control values of glucose (80 to 140 mg/dl) and ALP activity (44 to 147 IU/l) but they suffering from some kind of diseases. 3% had high SGL (140 ± 2.05 to 331 ± 1.63) while their ALP activity was normal. On the other hand 63% of the total patients showed high level of ALP activity (147 ± 1.24 to 444 ± 1.24 IU/l) but their SGL was quite normal. It was also found that 23% were those patients who had high value SGL and ALP. So this fraction confirmed that there is a biochemical relationship of SGL and ALP activity. Therefore, this study suggests that individuals with diabetes have a higher incidence of liver function test abnormalities than individuals who do not have diabetes.

Key words: Liver Function Tests • Alkaline Phosphatase • Diabetes • Serum Glucose

INTRODUCTION

Liver Function Tests (LFTs) are one of the most commonly requested screening blood tests and are used for the investigation of suspected liver disease, monitoring of disease activity, or simply as 'routine' blood analysis [1-3]. ALP is a hydrolase enzyme responsible for removing phosphate group (dephosphorylation) from many types of molecule, including nucleotides, protein and alkaloids [4-5]. Normal ALP levels in the adult are approximately 44 to 147 IU/L though levels are significantly higher in children and pregnant women [6]. Whenever liver cells or hepatocytes are damaged ALP is secreted in to blood. High level of ALP cause joint and bone disorders [7]. Along with other enzymes (SGPT and SGOT), ALP activity measurement in liver function test (LFTs) acts as a marker of liver disease/injury [8, 9].

Normal SGL ranges from 80 to 160 mg/dl. Glucose level is controlled by insulin hormone. This hormone is related to synthesis of glycogen in the liver when the

glucose level is increased after a meal and taken a large amount of carbohydrate and then gradually decreases the blood glucose level [10].

Any alteration in the liver may affect the glucose balance in the body. This is very common seen in the liver disease that glucose level is also increased [11]. Diabetes mellitus is chronic disease of impaired glucose/carbohydrate metabolism due to insufficient secretion of insulin [12, 13]. Many cells in the body have specific cell membrane insulin receptors. Insulin is responsible for utilization of glucose in these cells. Glucose transported in the muscle cell is regulated by insulin receptors [14]. There are several methods in literature for estimation of SGL and ALP but have different problem and disadvantages associated with them. Some are expensive; some are time consuming while some need very expensive instruments. Selectra E is a suitable method and was used in the DHQ hospital Gujranwala. We used selectra E instrument for estimation of SGL and ALP activity because it is cheap, less time consuming, has capacity to perform 50 tests at time and is fully automatic

computer system. Up till now literature does not show any data of correlation between SGL and ALP activity alone. So it was much more necessary to investigate association between SGL and ALP activity in different patients.

MATERIALS AND METHODS

Blood Sample Collection: Patients who visited outdoor clinics in the DHQ hospital for different diseases and their Doctors recommended them some kind of tests; they were taken to the sample collection room. 165 patients of mixed gender, age, profession and area were tested for only SGL and ALP activity during August 2014 (Fig. 1). A tourniquet was tied on the arm of patients and on the clear visibility of the vein; blood was drawn from the vein with the help of the BD syringe (3 ml) aseptically. 2.5 ml of blood was drawn from each patient, it was kept in the 5ml test tube and each test tube was marked with a number. After that, all these blood samples were kept at 4°C in refrigerator [15].

Centrifugation: Blood samples were centrifuged by the HP-BC screening device (BIOTECH) at the rate of 4000 rpm for 2 minutes. Blood changed into 2 layers. Upper yellowish layer was serum and the lower layer was containing blood cells in it. All of these test tubes were left for 15 minutes in order to settle down the moving elements [15].

Estimation of Alkaline Phosphatase: The serum samples were taken in the tray of selectra E machine for estimation of ALP using prescribed protocol. The selectra E machine is fully computerized and results were obtained on the monitor, then readings were noted.

Estimation of Sgl: For the estimation of glucose (random), serum of each blood sample was used. Supernatant from each test tube was carefully removed with the help of micropipette. 50 micro liter serum was taken from each test tube into a fresh eppendorf and was kept at proper place in Selectra E (which is a complete automatic machine) according to its sample number for the estimation of glucose at 37°C and 546 nm. Selectra E machine calculated the value of sugar in mg/dl [15].

Statistical Method: Important data were subjected to analysis of mean, variance and standard deviation (SD) using *Standard Deviation Calculator* “live”. The formula for Sample Standard Deviation was obtained from (<http://www.mathsisfun.com/data/standard-deviation-calculator.html>) [16].

RESULTS AND DISCUSSION

Data analysis and result interpretation are important parts of scientific research. Generalization and prediction cannot be achieved without these steps. The patients who visited the biochemistry lab in DHQ hospital were all out door patients (ODP) and were referred by the duty doctors. Most of the people were usually patients of different yet unknown diseases. These patients were of different age, sex, profession and area. Data that was collected had 88 male patients and 77 female patients (Figure 1 and 2). From which some patients were normal/control, some patients had high level of SGL while some are those who showed high level of alkaline phosphate only. But some patients had both glucose and alkaline phosphatase. This result showed that less female patients visited the Govt. hospitals than the male patients (Fig. 2).

Rural and Urban Patients: Out of total 165 patients involved in this study, 29 were rural and 136 were urban. From rural 15 patients have high level of SGL and alkaline activity (Fig. 3). Rural life is very hard and active. Most of the women suffer a double burden; they have to do domestic and field work. They are first rise and last go to bed. Also rural population starts treatment of diseases from their traditional old methods before they go to qualified Doctors [17]. Present study showed that the more urban people visited in clinical lab as compare to rural people. The patients who came from the rural had an end point of disease. This is due to less awareness in the rural areas where people suffering from serious disease, take light and severity of disease is increased in rural patients.

Suppression of insulin, increases glucose level in blood and reduces uptake of glucose by skeletal muscles [18]. In present study, 52% patients were of rural background and had high SGL and alkaline phosphate activity. The liver appears as the first point of contact for (and produces the initial immunological response to) bacteria and microbial components, polluted water, as well as other endogenous and exogenous toxins present in the portal blood [19]. Although rural people are more affected by liver diseases and diabetes which is more common in our society, yet rural people have a perfect diet and healthy environment. These results do not show that the disease is more common in rural areas. Note that only those patients who visited the hospital after severe disease were checked and involved in this study.

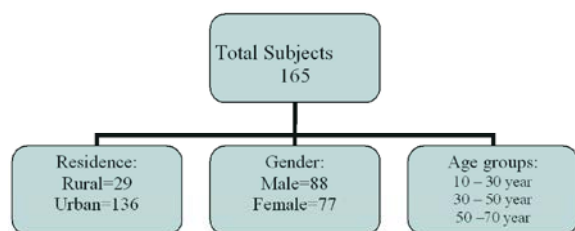


Fig. 1: Subjects involved in this study; Patient who visited outdoor clinics in DHQ hospital, District Gujranwala, Pakistan. Doctors recommended them for sugar test and LFTs.

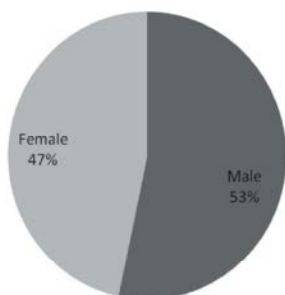


Fig. 2: Male and female patients by Percentage: The pie chart showing the participation of female and male patients by percentage and delivers the clear information that male were more in number and percentage then the female.

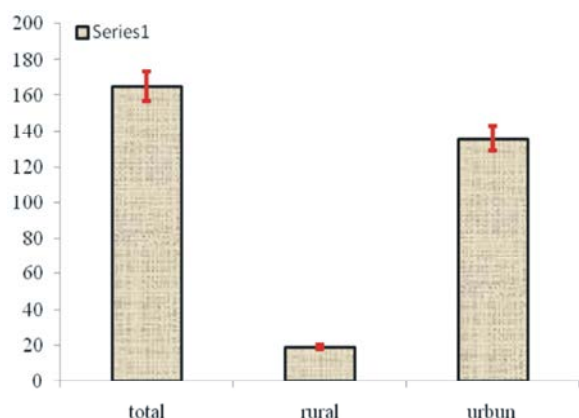


Fig. 3: Rural and urban patients: Total 165 patients from whom 29 are rural and 136 were urban. Red bar indicate the bar error with percentage.

Study of Abnormalities of SGL and ALP activity on the basis of different age groups Patients were separated into three categories according to their age. 33 patients ranged from 10- 30 year age, 105 patients ranged from 30 -50 year age, 27 patients range from 50 -70 year age and it was found that more patients (63.6%) of the age of 30-50 years visited civil hospital as compare to the other people in Gujranwala District (Fig. 4).

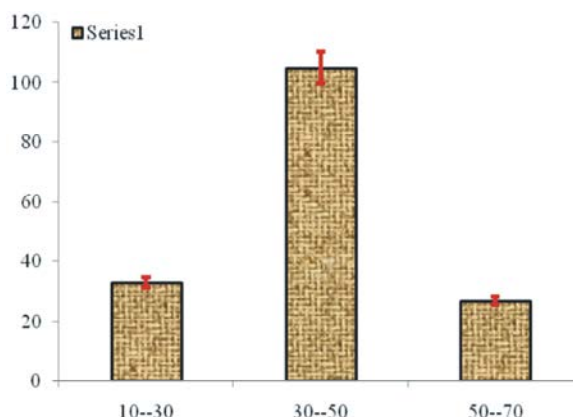


Fig. 4: Study of abnormalities on the basis of age groups; Patients were separated by their age three categories. Red bar indicate the bar error with percentage.

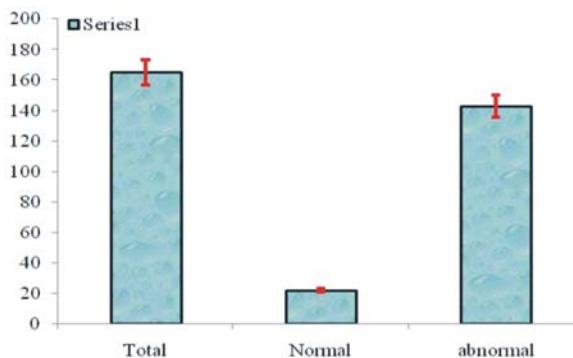


Fig. 5: Normal and abnormal patients with respect to SGL and ALP activity. Red bar indicate the bar error with percentage.

The disease caused in different groups of patients is random. Such people are self dependent and have a more facility to come in hospitals and old age people have high activity of ALP [20].

Normal and Abnormal Patients: Out of 165 patients, 9.7 % were normal or control (16 patients) with respect to SGL and ALP. Others were those whose alkaline phosphatase (ALP) and glucose level both were high (Fig. 5). Some were those of who only glucose level or ALP activity was high and others had high level of both SGL and ALP. This graph does not demonstrate that the liver disease is very common in our society. Studies are not related with some kind of survey where we visited door to door and checked the ALP and SGL. We checked only those patients who have some kind of disease and visited Doctors in DHQ hospital themselves.

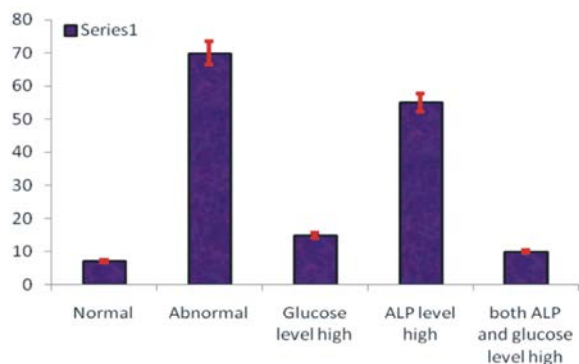


Fig. 6: Correlation between diagrams; the overall patients with normal and abnormal level. Red bar indicate the bar error with percentage.

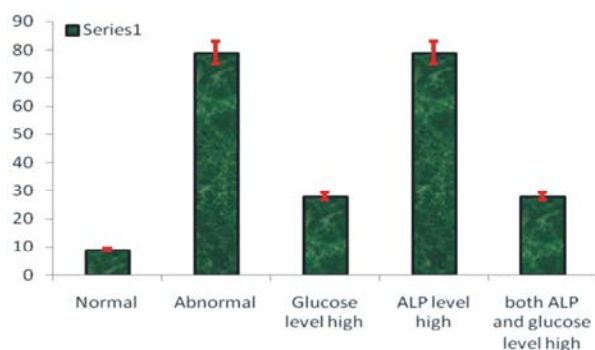


Fig. 7: Correlation diagrams of alkaline phosphate and glucose in male; Red bar indicate the bar error with percentage.

Correlation Between Alkaline Phosphate and Glucose in Female:

In another study only female patients were involved and it was found that out of 77 patients, 7 were normal (ALP =147 IU, glucose = 140 = 80 mg/dl) and 70 were patients which have high level of alkaline phosphatase and glucose level (Fig. 6). 15 patients were having high level of glucose only (glucose = 140 = 80 mg/dl) and 55 patients were having high level of ALP (ALP =147) from these patients there are 10 patients have high level of glucose and ALP (ALP =147, glucose = 140 = 80 mg/dl).

Correlation Between Alkaline Phosphate and Glucose in Male:

In males there are 88 patients out of which, 9 are normal (ALP 44 to 147 IU), glucose 80 to 140 mg/dl) patients and 79 remaining patients have random abnormal level of glucose and ALP (Fig. 7). 28 patients have high level of glucose (glucose = 140 = 80 mg/dl) and 79 patients have high level of ALP (ALP =147). From these total abnormal patients 28 patients are those which have high level of glucose and alkaline phosphate (ALP =147,

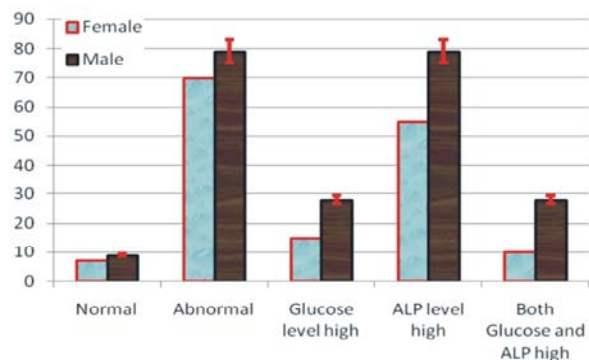


Fig. 8: Comparison between male and female Data for SGL and ALP activity; Red bar indicate the bar error with percentage. Comparisons with respect to level of glucose and activity of ALP in male and female patients.

glucose = 140 = 80 mg/dl). In present study 32% male patient confirmed a close association between abnormalities of SGL and ALP activity. Glucose level is high in males as shown in the figure 7 that normal patients are less in number, but the people who visited the clinical lab are mostly abnormal patients [21].

Comparison Between Male and Female Data:

Glycaemia represents the glucose level in the blood. Pathologically increase of the level of glycaemia occurs when the pancreas does not secrete enough insulin which may lead to diabetes and obesity. Long-term hyperglycemia also leads to cardio-vascular, neurological and liver complications [3]. Present study showed that glucose level was mostly high in male patients; alkaline phosphate was high in male and female patients (Fig 8). The results showed that there was significance difference between female and male on the basis of their age, sex, professions and the area of residency. The Data indicate that glucose and alkaline phosphatase level is more in male patients than female patients. The rural people are more seriously liver damaged as compare to the urban patients and abnormalities of SGL, ALP and other enzymes cause jaundice, encephalopathy, coagulopathy and circulatory dysfunction or even liver failure [22].

The fig. 8 show the comparison between the male and female patients, that male may attacked by diabetes and liver disease more compared to female patients. The one main reason of this unbalance contribution towards these diseases is that in our society the males do their jobs and remain under stress of social and economical factors. Mostly female remain in their houses and participate in managing the house and do a simple or easy life in the

houses. Females may face the less pollution and less hard working in daily routine life. Our result are similar to Kristine *et al.* [23] who reported that in first half of 20th century females were more effected by high level of glucose as compare to man but now the trend has been shifted that more males are suffering by high glucose level due to obesity [11], high contents of glucose used in food as well as socioeconomic tension [24]. Observation of sex difference, fat contact, insulin resistance and sex hormones may also enhance this notation. In a previous study of diabetes in the European patients, male patients were in excess than female population of European origin aged 15-40 years, with an approximate 3:2 male: female ratio. This ratio remained constant in young adults over two - three generations in some populations [24]. The results demonstrate that the level of diabetes is much higher in male than female patients. Male have great stress in their life and may face the pollution and disease causing agents from the external environment. Diabetes is more common in male and less seen in the female [11]. The level of ALP is also high in male than females. Results show that the ALP level is 14 % high in males as compare to female. The liver disease is more common in male patients. [25] studied the relationship between glucose and liver disease and they reported that there is some biochemical relationship between these diseases but the mechanism is unclear. One study showed that majority of the infected patients (80 %) who visit hospitals, develop chronic infection and are at high risk for end stage liver disease progression to enzymatic disorder, cirrhosis and hepatocellular carcinoma [2]. Hepatitis C virus (HCV) also causes persistent infection and is a major cause in the etiology of liver disorders like fibrosis, cirrhosis, hepatocellular carcinoma and abnormalities of SGPT, SGOT, SGL and ALP activities [26]. None of the individual test can predict the liver disease and at times it can lead to a confusing situation between liver disease and non-liver disease [27, 2]. The present study concludes that more sensitive and specific parameters are needed to correctly diagnose liver disease and at the same time it must rule out non-liver diseases [28]. Intense desertification, sustainable irreversible degradation of the environment and deterioration of living conditions caused an increased incidence of new socio-economic and environmental situations that require prevention and protection of the population living in ecologically unfavorable areas [29]. People with diabetes may also show liver diseases and patients with chronic liver disease also have high glucose level. In present investigation it was found that 38 patient out of 165 patients showed high level of both glucose and

ALP together. In other words 23% of the total patients showed higher values of both ALP activity and SGL therefore, confirmed a biochemical relationship between SGL and ALP activity.

CONCLUSION

The study showed that 23% of tested subjects have a high level of both glucose and alkaline phasphatase activity respectively. This finding showed biochemical association between the blood glucose and alkaline phosphatase activity. Urban people and middle ages (31-50 year) are careful about their health and show more frequency to visit hospitals. Malnutrition, polluted environment, polluted drinking water, burdon of workload, unsecure life due to terrorism and lake of plenty of rest are major causes of diabetes and liver disorders. There are more enzymes like SGOT and SGPT which may also be in biochemical relationship with SGL, therefore further research is required. The objectives of finding association of serum glucose and ALP were achieved successfully and more studies are suggested.

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