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RESEARCH ARTICLE

Serum amylase and lipase activities in newly diagnosed patients with type 2 diabetes mellitus.

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Abstract

Background: Diabetes mellitus is caused by a derangement in the secretion or function of the endocrine portion of the pancreas. There is a close anatomical and functional relationship between its endocrine and exocrine portions. To address this issue the current study was designed to evaluate the blood glucose and amylase and lipase levels of diabetic patients as representatives of the two portions of the pancreas respectively.
Methods: The study was carried out on 75 newly diagnosed patients of type 2 Diabetes Mellitus.50 age and sex matched healthy individuals were enrolled as controls. Fasting venous blood samples were collected from both cases and controls and were analyzed for blood glucose, serum amylase, serum lipase and lipid profile (total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein) by standard methods.
Results: Significantly (p<0.001) low serum amylase activity and significantly high (p<0.001) serum lipase activitywas found in diabetics as compared to controls. Also total cholesterol and HDL(high density lipoprotein) were significantly lower in diabetic patients(p<0.001).
Conclusion: From our study it was concluded that in Type 2 diabetes, amylase activity decreased and lipase activity was raised which highlighted the interactions between the two functional entities of the pancreas. Diabetes which affects the endocrine pancreas could also affect the exocrine part due to the close structural vicinity between the two.

Introduction:-

The global prevalence of diabetes and especially type 2 diabetes mellitus (T2DM) is increasing at an alarming rate. According to the recent update by the International Diabetes Federation(IDF) more than 382 million adults aged 20-79 years had diabetes in 2013. Epidemiological data indicate that the prevalence of diabetes has been rising in the South-East Asian countries for at least two decades and current estimates have surpassed all previous predictions [1]. India, the largest country in the Region, has more than 65 million adultswith diabetes and has the second highest number of cases in the world after China[2].The dramatic economic changes have had a great impact on urbanization and lifestyle of the Indians, which together with genetic predisposition contributed to the rise in diabetes in India[3].

The main pathophysiological feature of T2DM is impaired insulin secretion and insulin resistance, so it is characterized by hyperglycemia[4,5,6]. Insulin resistance is defined as reduced sensitivity of targetorgan to the biological effects of insulin[7]. Multiple defects in insulin secretion and signaling in T2DM may affect the enzyme synthesis and release from exocrine pancreas[8]. The pancreas serves dual functions as a digestive organ and as an endocrine organ, by secreting digestive enzymes like amylase and lipase and endocrine hormones [9].
For many years, low serum amylase was thought to reflect diffuse pancreatic destruction secondary to advanced pancreatic diseases, such as chronic pancreatitis [10]. Recently, several large clinical studies have shown that low serum amylase is also associated with metabolic syndrome and Diabetes[11, 12].

Pancreas is also the primary source of serum lipase. Increased activities of serum lipase have also been associated with pancreatitis, pancreatic duct obstruction, pancreatic cancer, kidney disease, and other pancreatic disease[13, 14].

Within the pancreas, which is a mixed exocrine-endocrine gland, the exocrine portion makes up the greatest volume (84%). Ductal cells and blood vessels make up around 4% of the volume, while the endocrine part makes up 2% of the volume. The other part (10%) is occupied by an extracellular matrix. The acinar tissue in the pancreas is in close vicinity of the islets. Because of this close morphological relationship, functional interactions are likely to occur between the exocrine and endocrine pancreas in any disease, which affect this organ[15].

In type 2 Diabetes mellitus, the continuous interstitial matrix connection between the endocrine and the exocrine pancreas is lost in animal models and humans, which results in a dysfunctional insulino-acinar-ductal-incretin gut hormonal axis[16]. Besides these putative mechanisms, there are multiple defects in the insulin secretion and the signaling in type 2 diabetes, which may affect the enzyme synthesis and release in the exocrine pancreas[6].

In recent years, many studies have provided evidence that digestive organs contribute to the control of energy balance and glucose homeostasis via gut hormones[17]. The exocrine-endocrine relationship in the pancreas has been a focus of much attention in animal and cellular studies[9]. On the other hand, few clinical studies have been conducted and the clinical relevance of low serum amylase levels remains unknown, although the impact of high serum amylase levels has been investigated by numerous clinical researchers in terms of acute pancreatitis.To date, the clinical relevance of low serum amylase levels remains poorly understood. Animal and cellular studies regarding the relationship between the endocrine and the exocrine pancreas have consistently showed that insulin affects basal and stimulatory amylase secretion via the islet-acinar axis. Briefly, insulin binds to its receptor on acinar cells and stimulates amylase secretion through various pathways [18,19,20]. However, the nature of this relationship between low serum amylase and clinical conditions has been addressed by relatively few, small-scale human studies that yielded some conflicting results [21,22].

With this background, the present study was conducted to evaluate serum amylase along with serum lipase activity in type 2 diabetes mellitus in order to elucidate a possible correlation between the functional entities of the pancreas.

Material and Methods:-
The present study was conducted in the Department of Biochemistry, Assam Medical College and Hospital, Dibrugarh, Assam, India after obtaining prior permission from the Institutional Ethical Committee. The study was carried out for a period of 6 months.75 newly diagnosed patients of type 2 Diabetes Mellitusbetween the age-group of 30-60 years, of both sexes, attending the Diabetic Clinic under the Department of Medicine, were included as cases and were compared with 50 age and sex matched healthy individuals. Patients with history of cardiovascular disease, chronic renal insufficiency and alcohol abuse and those currently on medication for hypertension, hypercholesterolemia were excluded from the study. All the subjects, including the controls were fully informed about the study and their voluntary informed consents were taken.Detailed history was obtained. Fasting venous blood was collected from the cases (diabetic patients) as well as the controls.It was centrifuged(at 3000 rpm for 10 minutes) and the plasma/serum was separated for analysis on the same day for the blood glucose, the lipid profile which included the estimation of serum triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and serum amylase and serum lipase. The sample analysis was carried out on a fully automated Siemens Dimension RxL analyzer by using different reagent kits as per the procedure which was defined by the manufacturer. The estimation of fasting blood glucose was done by the glucose oxidase-peroxidase method. The serum triglycerides were measured by the enzymatic (GPO–PAP) method. For the determination of the total cholesterol, an enzymatic (CHOD–PAP) colorimetric method was used. The direct measurements for LDL and HDL were done by using enzymatic methods. The amylase activity was measured by a photometric enzymatic method (AMY, CNPG3 METHOD).The lipase activity was measured by a colorimetric method (LIPL METHOD).The results were analyzed statistically by using the Student's t test and Pearson's correlation coefficientusing Graph Pad Prism 5. Statistically $p < 0.05$ was considered significant, $p < 0.001$ highly significant, while $p > 0.05$ was considered not significant. The results of the study were expressed as Mean ± S.D.

Results:-
Diabetes is a global problem and with an increasing incidence worldwide, it would be the leading cause of morbidity and mortality in the future. Diabetes is invariably associated with a derangement in the functions of the pancreas.

The pancreas has dual functions in our body, as a digestive organ and as an endocrine organ. To evaluate this complex relationship between the two functional entities of this organ, the present study was conducted. 75 newly diagnosed cases of Type 2 diabetes **(group 1**) and 50 controls, **[group 2 (healthy subjects)]** were selected to assess the possible exocrine-endocrine relationship of the pancreas, by evaluating the serum amylase and lipase levels and the blood glucose in the two groups. The other biochemical parameters like the lipid profiles of the cases were compared with those of the healthy controls and they were also correlated with the amylase and lipase activities in the two groups.The following were the findings of this case–control study. The clinical characteristics of the study subjects are shown in Table 1.

Table 1:- clinical characteristics of the study subjects.

Characteristics of the subjects	Group ICasesn=75(Mean ± S.D)	Group IIControlsn=50 (Mean ± S.D)	p value
Age(years)	**45.73 ± 6.13**	**44.54 ± 7.29**	**0.326**
Sex (males: females)	**46:29**	**29:21**	**0.709**
FBG (mg/dl)	**162.6 ± 45.46**	**93.12 ± 9.63**	**<0.001***
TGL (mg/dl)	**136.9 ± 84.53**	**127.0 ± 74.17**	**0.502**
Total cholesterol (mg/dl)	**121.2 ± 20.94**	**146.3 ± 14.4**	**<0.001***
(HDL)(mg/dl)	**42.75 ± 13.38**	**70.98 ± 15.61**	**<0.001***
(LDL) (mg/dl)	**51.24 ± 14.46**	**48.51 ± 11.64**	**0.268**
Serum amylase (U/L)	**47.95 ± 23.63**	**107.1 ± 37.97**	**<0.001***
Serum lipase (U/L)	**212.5 ± 106.6**	**123.6 ± 33.35**	**<0.001***

*$p<0.001$ is highly significant
FBS = Fasting Blood Glucose, TGL = Triglycerides, HDL =High density lipoproteins,
LDL = Low density lipoproteins

The mean ages in the diabetic group (**45.73 ± 6.13 years**)and in the healthy control group (**44.54 ± 7.29 years**) were comparable and their difference was not significant. Out of 75 cases enrolled for the study 61% were males and 39% were females**[Figure 1]** which was comparable to the 58% males and 42% females in the control group of 50 individuals **[Figure 2].**
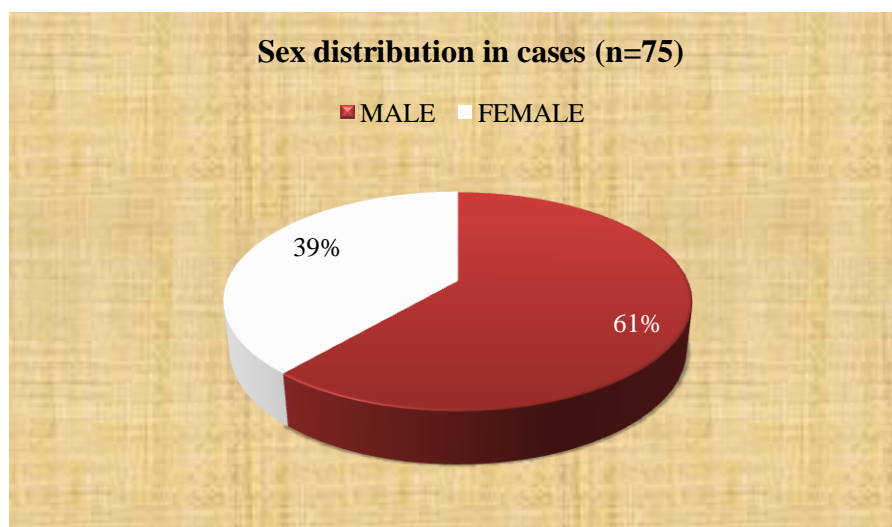

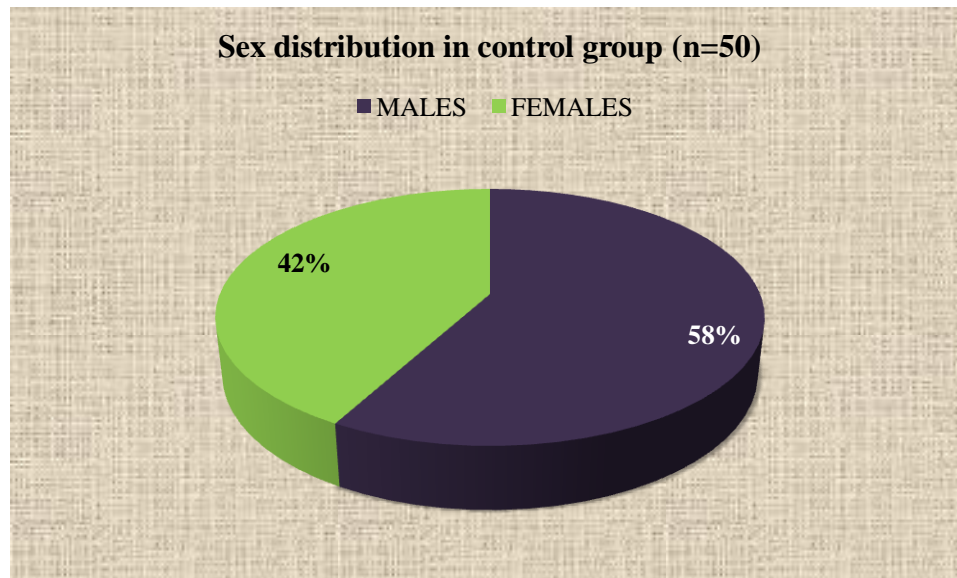**Figure 1:-** Distribution of diabetic cases based on gender (males/females)

Figure 2:- Distribution of controls based on gender (males/females)

Amongst the cases, 42.7% were between 40-49 years of age,34.6% were 50 years and older and the remaining 22.7% were between 30-39 years of age as shown in **Figure 3**.
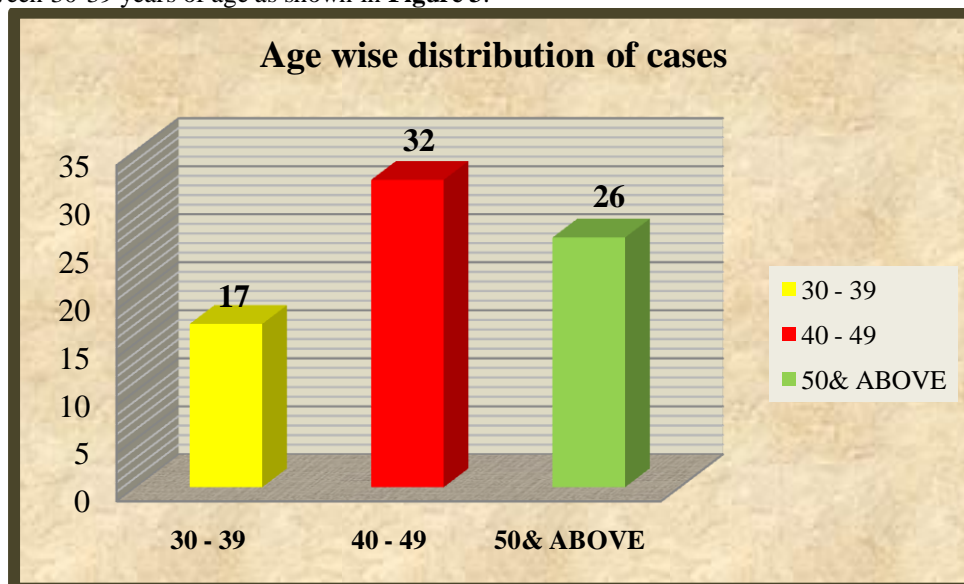

Figure 3:-Distribution of cases according to different age groups

Figure 4 shows that the mean (±SD)fasting blood glucose levelsof cases **(162.6 ± 45.46 mg/dl)**was significantly (**p <0.001**) higher as compared to the controls **(93.12 ± 9.63 mg/dl).**
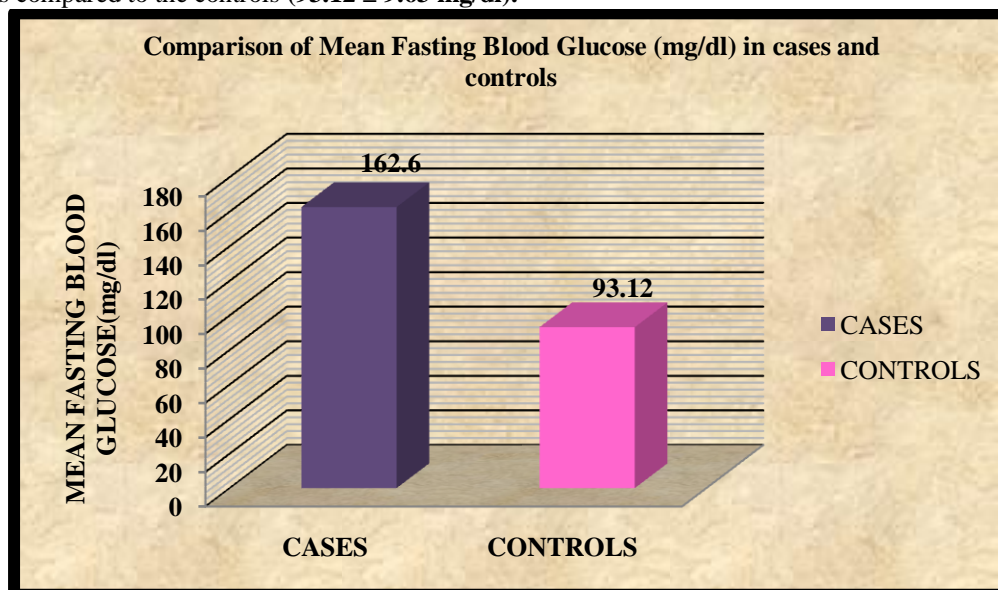

Figure 4:-Comparison of mean fasting blood glucose levels of diabetics with that of healthy controls

Figure 5 shows that the serum amylase activity in the diabetic group (**47.95 ± 23.63 U/L**) was significantly (**p<0.001**) low compared to that in the controls (**107.1 ± 37.97 U/L**).On the other hand, the serum lipase activity (**212.5 ± 106.6 U/L**) in the diabetic group was significantly (**p <0.001**) raised in comparison to that of the control group (**123.6 ± 33.35 U/L).**
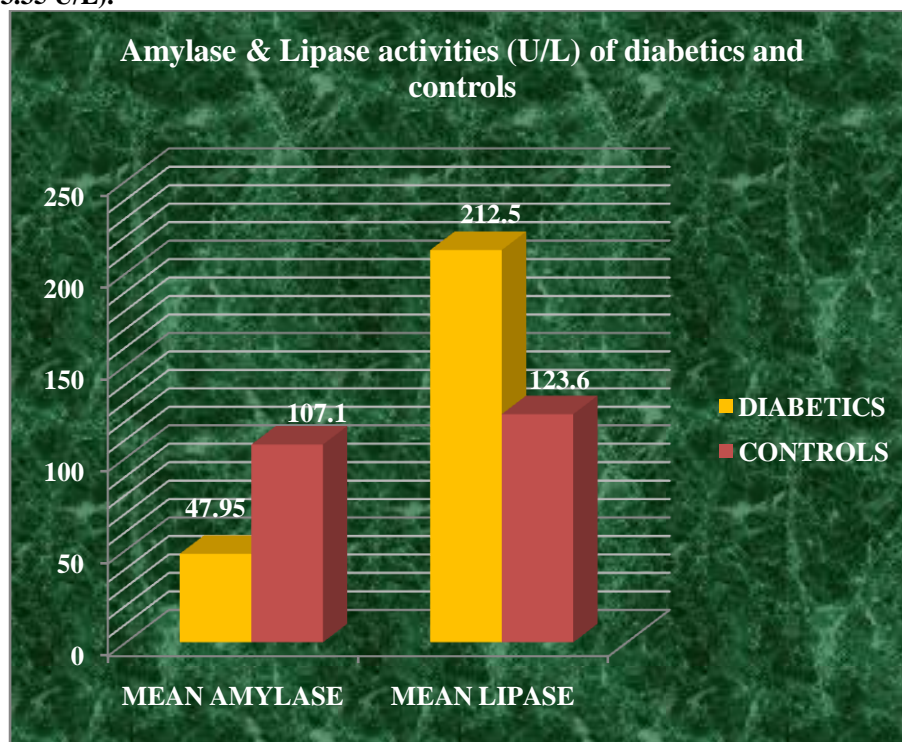

Figure 5:-Comparison of mean serum amylase and lipase activities in diabetics and healthy controls

Total cholesterol and HDL were significantly lower **(p<0.001)** in the diabetic group in comparison to the control group.LDL levels were higher in the diabetic group (**51.24 ± 14.46 mg/dl**) than in the controls (**48.51 ± 11.64**

mg/dl) but the difference was not statistically significant. This lowering of cardio-protective factors like HDL and the increase in LDL consolidated the well-known risk of cardio-metabolic abnormalities in diabetic individuals.

It was found that there was a negative correlationbetween serum amylase activity and fasting blood glucose levels in the diabetic patients (**r= -0.152**) which was not significant (**p= 0.192**).There was also no significant correlation of serum amylase with Total cholesterol, triglycerides, HDL and LDL.

Interestingly, serum lipase activity showed significantly (**p <0.001**) positive correlation (**r = 0.617**) with fasting blood glucose levels in the diabetic groupas shown by **Figure 6** below.On the contrary, no significant correlation could be found between lipase activity and lipid profile in the diabetic subjects.
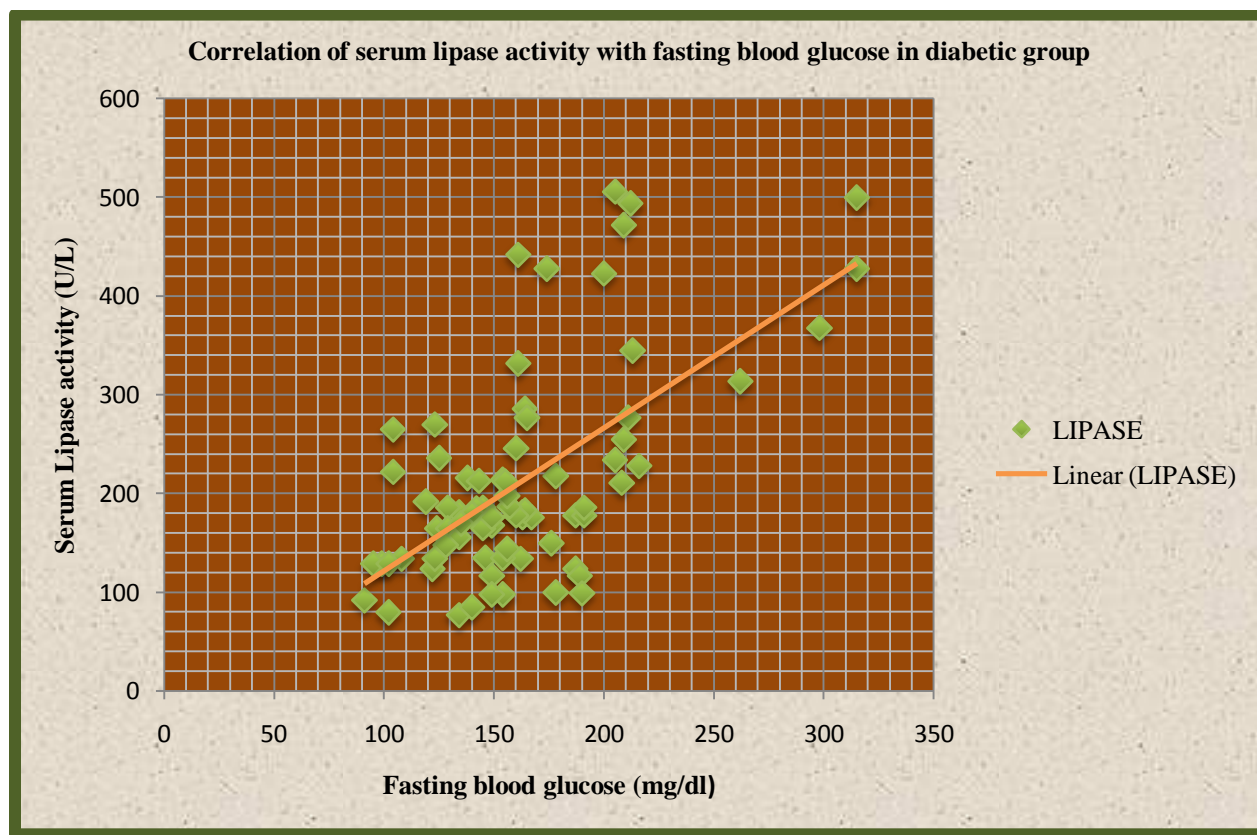

Figure 6:-Correlationof serum lipase activity (U/L) with fasting blood glucose (mg/dl) in diabetic group

Discussion:-

The mean (±SD)age of diabetic patients in our study was 45.73 ± 6.13years. Similar to our findings, Swati Pathak et al.(2015)[23] in their study found the mean age in diabetics to be 54.12 ± 10.4 years and Reena Jain et al.(2014)[24] found mean age group in diabetic patients to be 53.41±10.48 years which further consolidates the fact that type II diabetes primarily affects adults above 40 years of age.

The mean (±SD) fasting blood glucose level of cases (**162.6 ± 45.46 mg/dl**) was significantly (**p<0.001**) higher as compared to that of controls (**93.12 ± 9.63 mg/dl).**These findings were consistent with that of the studies by Hattf et al.(2014)[25], Annette et al.(2003)[26]& Tan et al.(2001)[27]**.**

We found in our study a significantly low amylase activity in the diabetic group as compared to that in the healthy controls. There is evidence that the pancreatic hormones, insulin and glucagon, influence the enzyme synthesis and release in the exocrine pancreas. Insulin has a trophic effect on the acinar cells, whereas glucagon has been found to have an inhibitory influence on the exocrine secretions and moreover, there is a decrease in the sensitivity of the diabetic pancreatic acini to secretagogues. So, the insulin deficiency and the glucagon excess in diabetes affect the normal milieu of the pancreas, thereby decreasing the total volume, the amylase secretions and the bicarbonate content of its exocrine secretions [15].

Moreover about 50% of the diabetics have pancreatic fibrosis and other pathological findings such as atrophy, fatty infiltration and loss of the exocrine acinar cells[28, 29].Also, diabetic neuropathy may lead to impaired entero-pancreatic reflexes and exocrine dysfunction[30].Elevated hormones and peptide concentrations (glucagon, pancreatic polypeptide P, somatostatin) may suppress the exocrine function[9, 17, 31].

It has been added that cytokines such as TGF-beta 1, TGF-alpha (transforming growth factors) and TNF-alpha (tumor necrosis factor), gastrin and low regulatory gene functions may interact and further impair the exocrine and endocrine functions[32, 33, 34].

Our results suggest that the low serum amylase levels in diabetes are associated with an impaired insulin action due to insulin resistance and/or inadequate insulin secretion, as was indicated by the raised blood glucose levels in our study. Similar findings were also observed by Reena Jain et al.(2014)[24]. However in contrast to our findings, Hattf et al.(2014)[25] found significant increase in serum amylase activity in diabetic group (175.35 ± 21.74 U/L) compared to the control group (40.19 ± 10.50 U/L).Insulin functions prevent uncontrolled triglyceride hydrolysis and limit gluconeogenesis[35, 36]. Decreasing levels of insulin in type 2 diabetes mellitus can cause uncontrolled triglyceride hydrolysis and cause the free fatty acid (FFA) levelsto increase by increasing the secretion of lipase from exocrine pancreas. This increased FFA concentration will reduce the inhibiting effect ofinsulin upon the gluconeogenesis and glycogenolysis and thereby the blood glucose value will also rise. This fact was consolidated by our study where we found significantly higher lipase activity in the diabetic group compared to the controls. In addition serum lipase activity showed significant positive correlation with increasing fasting blood glucose levels in the diabetic patients which was comparable to the findings of the study conducted by Arie Srihardyastutie et al. (2015)[37].

Conclusion:-
Therefore to conclude, significant decrease in amylase activity and increase in lipase activity in patients with Type 2 Diabetes Mellitus could indicate a supposed interplay between the endocrine and the exocrine functions of the pancreas owing to their structural vicinity. This fact can only be established after trials, preferably for longer duration with larger sample size and with additional investigations like fasting plasma insulin along with adequate consideration to other pancreatic pathologies.

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