Alpha-amylase Activity in Serum is Positively Associated with C-reactive Protein in Obesity and Diabetes

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ABSTRACT: Alpha-amylase plays a critical job in metabolic homeostasis. Appraisal of alpha-amylase activity in metabolic disorders, for example, obesity and diabetes are important. The current investigation was meant to evaluate the relationship of alpha-amylase activity with C-reactive protein in obesity and diabetes. Alpha-amylase activity along with different biochemical markers like glucose level, triglyceride, total cholesterol, C-responsive protein, and creatinine level were analyzed in healthy, obese, and diabetes populations. In obese and diabetes, a significant deviation ($p<0.05$) was seen in the degree of biochemical markers including blood glucose, lipid profile, C-reactive protein (CRP), creatinine, and alpha-amylase action when compared with healthy volunteers. The alpha-amylase activity was found to be strongly associated ($p< 0.01$) with Body Mass Index (BMI), blood glucose level, and duration of diabetes Thus, it can be stated that alpha-amylase can initiate a cross-linking mechanism between BMI and blood glucose level facilitating the propensity of obesity and diabetes. Moreover, alpha-amylase indicated a positive correlation with CRP, a marker for inflammation, proposing a complex job in mediating inflammation in obesity ($r= 0.486$, $p < 0.01$) and diabetes ($r=0.507$, $p<0.01$). Serum creatinine was demonstrated as an insignificant positive correlation with alpha-amylase activity in obesity and type-2 diabetes. Alpha-amylase can be considered as a significant hazard factor in the pathogenesis of obesity and diabetes-related complications.

Key words: alpha-amylase; C- reactive protein; Obesity; Diabetes; Zinc

INTRODUCTION

Obesity is a public health concern that inclines the danger of numerous interminable ailments like type 2 diabetes, cardiovascular diseases, hypertension, malignancy, and so forth.[1] Obesity has been characterized as the excess accumulation of fat in adipose tissue. Adipose tissue not only serves as an energy depot but also secretes distinct pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF-alpha), adiponectin, resistin affects the vascular endothelium, glucose, and lipid metabolism.[2-4] Recent research has identified that the inflammatory reaction is a typical factor among obesity, diabetes, and cardiovascular diseases.[5,6] C-reactive protein (CRP), an inflammatory biomarker, is produced by hepatocytes under the stimulation of inflammatory cytokines.[7] The elevated level of CRP has been documented with the increase in body mass index (BMI) in children and adolescents.[8] Matull *et al* reviewed that, serum amylase activity is commonly monitored as a biomarker for pancreatic inflammation.[9] It has been reported that, in the case of obesity, glycogen is metabolized by pancreatic and salivary alpha-amylase and leads to increased blood glucose.[10] The role of alpha-amylase activity in type 2 diabetes and metabolic syndrome is controversial. A study by Shankaraiah and Reddy,[11] reported that post-prandial alpha-amylase activity was significantly higher in type 2 diabetes compared to normal control in the Indian ethnic group whereas, Nakajima *et al*, demonstrated that low serum alpha-amylase activity is associated with the metabolic syndrome and diabetes.[12]

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Trace elements are present in a minor amount in the body but play an important role in different enzyme actions. The biological role of zinc has been studied in cellular metabolism and immunological responses.[13,14] Recently we found an inverse association of elemental zinc and C-reactive protein in chronic liver disease patients.[15] Albeit a few investigations had been directed to investigate the role of alpha-amylase activity and CRP independently yet no endeavor has been taken to relate these parameters in obesity and diabetes. Furthermore, little information about the governing role of zinc in alpha-amylase activity is available. Taking all these things into consideration, we aimed to study the association of alpha-amylase activity with CRP in obesity and diabetes along with to let know the effect of elemental zinc in alpha-amylase activity in ex vivo.

MATERIALS AND METHODS

Study design. This case-control study was carried out with adult patients attending the Diabetes Hospital, Noakhali, Bangladesh. Patients and control groups were matched by age, sex, and socioeconomic conditions. Children, non-cooperative volunteers, and volunteers having comorbid diseases and mental disorders were excluded from the study. Detailed patient history was taken with a predesigned questionnaire. Every volunteer was informed about the purpose of this study and written consent was taken before data collection. Ethical considerations have been reviewed (Ref: 20/2020) by the Institutional Ethical Clearance Committee of Noakhali Science and Technology University, Bangladesh.

Sample size determination. Sample size was determined using the following statistical equation.

$N = \{z^2 X p(1-p)\}/e^2$

Considering 95% CL (z=1.96), 5% Error margin (e=0.05) and 5% disease prevalence (p=0.05), estimated samples size is 73. But we get response from 30 patients to collect blood. Number of healthy volunteers were matched with disease volunteers. Finally, thirty healthy volunteers as control, thirty diabetes and thirty obese patients were recruited in this study.

Collection of blood and processing. Fasting venous blood was drawn from each participant. The samples were allowed to clot within 30 min, and after centrifugation, serum was collected. Extracted serum stored at −80 °C. All the steps were carried out in an aseptic condition to avoid possible interference in the test readings.

Biochemical analysis. These samples were employed for determining the serum alpha-amylase activity (Merck, Germany, Cat# MAK478), serum blood glucose (GlucoLeader, HMD Biomedical Inc, Taiwan, Cat# 641), serum triglyceride (Linear Chemicals, Spain, Cat#1155017), serum cholesterol (Linear Chemicals, Spain, Cat#1118017), C-reactive protein (Anamol Laboratories, India, Cat# LX-02), and creatinine (Randox Laboratories, UK, Cat# CR510) using the respective assay kit according to the manufacturer's instruction and applied in our previous research.[15]

Statistical evaluation. All results were expressed as mean ± SEM. The correlation coefficient was measured to test a positive or negative linear relationship between two variables. Students 't' test was applied for statistical analysis. Values $p < 0.05$ was considered as statistically significant.

RESULTS

Selection of volunteers. Characteristics of the study population have been described in table 1. Total ninety volunteers were recruited in this study, All the groups i.e control, obese, and diabetes contained thirty volunteers each. Study groups were matched by age. Obese subjects showed significantly ($p < 0.05$) higher BMI (30.01 ± 0.72 kg/m^2) than that of control (23.99 ± 0.43 kg/m^2) but there were no significant different in BMI for control and diabetes (23.64 ± 0.27 kg/m^2) group. On the other hand, the diabetes group showed statistically ($p <0.05$) elevated fasting blood glucose level (9.73 ± 0.89 mmol/l) than that of the control (6.76 ± 0.19 mmol/l) but the obese group (6.97 ± 0.22 mmol/l) did not showed any

significant changes. These demographic data demonstrated a qualified selection of population involved in this study.

Biochemical assessments. The findings of the assessed different biochemical parameters are shown in table 2 and figure 1.

Alpha amylase activity. The alpha-amylase is responsible for the breakdown of polysaccharide. Its activity in obese and diabetes were studied. A significantly higher ($p<0.05$) activity of alpha-amylase were recorded in obese and diabetes compared with healthy control (figure 1) in this study.

Table 1. Characteristics of study population.

Parameter	Control (n = 30)	Obese (n = 30)	Diabetes (n = 30)
Age (yr)	43.3 ± 10.28	45.5±12.34	47.6 ± 11.52
BMI (kg/m^2)	23.99 ± 0.43	30.01 ± 0.72*	23.64 ± 0.27
Fasting blood glucose level (mmol/l)	6.76 ± 0.19	6.97 ± 0.22	9.73 ± 0.89*
Post prandial glucose level (mmol/l)	ND	ND	14.58 ± 0.89

ND, not done; *BMI* body mass index; Values are presented as mean ± SEM, Students 't' test was applied for statistical analysis, *p < 0.05 versus control

Table 2. Serum biochemical assay.

Parameter	Control (n = 30)	Obese (n = 30)	Diabetes (n = 30)
Triglyceride (mg/dl)	161.72 ± 6.14	193.47 ± 9.97*	291.89 ± 31.41*
Total cholesterol (mg/dl)	170.32 ± 7.28	180.40 ± 4.78*	182.77 ± 8.15*
C-Reactive protein (mg/dl)	4.2 ± 0.94	4.4 ± 0.98	11.18 ± 2.50*
Creatinine (mg/dl)	1.89 ± 0.15	2.5 ± 0.12*	1.97 ± 0.17

Values are presented as mean ± SEM, Students 't' test was applied for statistical analysis, *p < 0.05 versus control

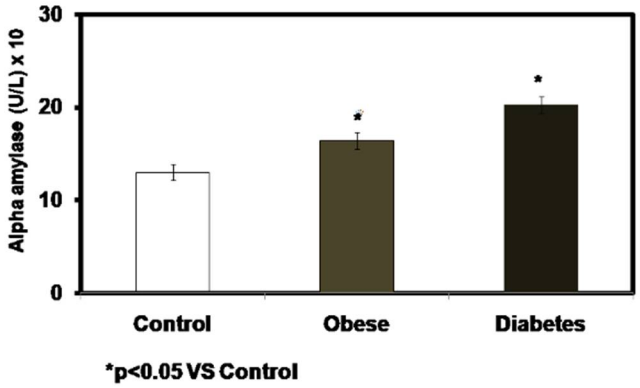

Figure 1: Alpha amylase activity measured in study population.

Serum cholesterol and serum triglyceride. Serum cholesterol and serum triglyceride were assessed in this study. As predicted, significant ($p < 0.05$) increase in serum cholesterol (180.40 ± 4.78 mg/dl for obese and 182.77 ± 8.15 mg/dl for diabetes VS 170.32 ± 7.28 mg/dl for healthy control), serum triglyceride (193.47 ± 9.97 mg/dl for obese and 291.89 ± 31.4 mg/dl for diabetes VS 161.72 ± 6.14 mg/dl for healthy control) were recorded.

Serum C-reactive protein. As a predictor for inflammatory response in obese and diabetes, serum C-reactive protein (CRP) were evaluated. Relatively

higher serum CRP have been accounted for obese (4.4 ± 0.98 mg/l) but a statistically significant ($p < 0.05$) elevated serum CRP were estimated in diabetes (11.18 ± 2.50 mg/l) while compared with healthy control (4.2 ± 0.94 mg/l).

Serum creatinine. In the case of serum creatinine, significant elevation ($p < 0.05$) was recorded in obese persons (2.5 ± 0.12 mg/dl) but diabetes (1.97 ± 0.17 mg/dl) showed an insignificant increase than that of control (1.89 ± 0.15 mg/dl).

Zinc sulfate reduces alpha-amylase activity. As a matter of interest, we incubated the serum with different doses of zinc sulfate and estimated the alpha-amylase activity and we found that zinc sulfate was able to reduce the alpha-amylase activity dose-dependently. Data present in table 4.

Table 3. Pearson's Correlation with alpha amylase activity and other parameters.

Parameter	Healthy		Obese		Diabetes	
	r	P	r	P	R	P
BMI	0.854	<0.01*	0.704	<0.01*	0.882	<0.01*
Triglyceride	0.139	0.23	0.931	< 0.01*	0.638	<0.01*
Cholesterol	0.425	0.05*	0.850	0.04*	0.737	0.03*
Blood glucose (Random)	0.157	0.20	0.686	<0.01*	0.825	<0.01*
Creatinine	0.315	0.05*	0.249	0.1	0.181	0.17
CRP	0.329	0.04*	0.486	<0.01*	0.507	<0.01*

*Statistically significant, p <0.05

Table 4. *ex vivo* Inhibition of alpha amylase activity by Zinc sulfate.

Group	Without treatment	Treatment (0.5 mg/ml)	Treatment (1 mg/ml)
Obese	131.67 ± 2.07	65.84 ± 1.04*	62.84 ± 1.65*
Diabetes	137.65 ± 0.34	71.8 ± 2.19*	47.9 ± 1.47*

Values are presented as mean ± SEM, Students 't' test was applied for statistical analysis * p <0.05 versus without treatment

DISCUSSION

The predominance of obesity is pandemic all through the world. As indicated by a report of the World Health Organization, more than 2 billion adults were estimated as overweight of whom 670 million considering as obese in the year 2014. If the trend is going on by 2025 about 2.7 billion and as well as 1 billion adults worldwide will be affected by overweight and obesity and about US$ 1.2 trillion will cost per year to treat obesity-related consequences by 2025 as per the prediction of World Obesity Foundation.[16,17]

Obesity and diabetes are closely associated. Here we studied the association of alpha-amylase activity with C-reactive protein along with the propensity of obesity and diabetes. Alpha-amylase is the major form of amylase enzyme in humans and other animals. It is responsible for the breakdown of 1,4-a-D-Glucosidic linkage in polysaccharides. Alpha-amylase activity in this study population has been presented in figure 1. In this study, alpha-amylase activity in obese and diabetes was significantly greater than that of control ($p < 0.05$). Diabetes is having more activity than obese. Hiriri *et al.*[10] reported that, in obesity, glycogen is metabolized by amylase and causes the increase in blood glucose levels in obesity and diabetes. As of intrigue, we assessed the correlation between the alpha-amylase activity and BMI in obese and diabetes, and a strong positive association was found ($r = 0.704$, $p < 0.01$ and $r = 0.882$, $p < 0.01$, respectively). A similar positive association with alpha-amylase activity was observed for triglyceride, total cholesterol, random blood glucose measured in obese and diabetes. Data presented in table 3. Taking all these data it is evidenced that, serum alpha-amylase activity was positively associated with obesity and diabetes. Our

finding is in accordance with the report from Shankaraiah[11], where they described the higher alpha-amylase activity is positively linked with diabetes in the Indian population. Furthermore, inhibition of digestive enzymes like alpha-amylase in the management of obesity and diabetes has been reviewed and well documented.[18] The inhibition of alpha-amylase controls the postprandial glucose level in obese and type-2 diabetic patients.[19]

Obesity and diabetes are considered low-grade inflammation. We estimated the serum CRP as a marker for inflammation. Serum CRP synthesized by the liver after the stimulation of Interleukin-6 (IL-6) markedly increased within hours of infection or inflammation.[7] A little data is available for the CRP in association with alpha-amylase activity in obesity and diabetes. Our data presented in table-2 showed a statistically elevated CRP level in obesity and diabetes patients compared with healthy control. Similar data have been reported in the Korean diabetes population by Kanmani *et al.*[20] Moreover, diabetes patients showed a more elevated CRP level than that of the obese population in our study. Of interest, we aimed to correlate between alpha-amylase activity and CRP in obese and diabetes. Moderate positive association was found between alpha-amylase activity and CRP in both obese ($r= 0.486$, $p < 0.01$) and diabetes ($r=0.507$, $p<0.01$). The serum activity of alpha-amylase is monitored in pancreatitis.[9] Pancreatitis is an inflammatory condition, where the increased level of CRP is reported.[21] Thus, it is not unlikely in case of obesity and diabetes, where the increased alpha-amylase activity is positively associated with an elevated level of CRP. This data positively supports the notion of the inflammatory state of obesity and diabetes.

Previously we reported an inverse association of Zinc, a trace element, with CRP in an inflammatory disease like chronic liver disease.[15] Hence, here we aimed to investigate the effect of elemental zinc on alpha-amylase activity. We incubated the serum from study groups in the presence and absence of zinc sulfate (0.5 mg/ml and 1.0 mg/ml) for 30 min at 37^{o}C. After the incubation period, we measured the alpha-amylase activity. We found a dose-dependent inhibition of alpha-amylase activity in the presence of Zinc sulphate. Data are presented in table 4. Although this study did not capture the effect of Zinc Sulphate *in vivo*, this *ex vivo* analysis suggested that elemental zinc like Zinc sulfate might have inhibitory action for alpha-amylase activity. A recent study by Liao *et al.* uncovered the inhibitory action of alpha-amylase activity by zinc.[22]

In conclusion, our data presented here, demonstrate the positive association of alpha-amylase activity with CRP in obese and diabetes populations. Secondly, elemental zinc may be responsible for the inhibition of alpha-amylase activity in vivo. Although a large-scale study is essential to get the insight effect of zinc in the alpha-amylase activity.

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