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EXOGYCOSIDASES, PROTEIN-BOUND SIALIC ACID AND CARBOHYDRATE DEFICIENT TRANSFERRIN AS ALCOHOL BIOMARKERS : A CORRELATIVE STUDY OF BLOOD AND SALIVA IN ALCOHOL DEPENDENT MALES

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ABSTRACT

BACKGROUND : Identification of alcoholics especially in early stages of alcohol abuse is crucial in preventing adverse health effects and social consequences. Various biochemical parameters in blood have been proposed as alcohol biomarkers. study alcohol biomarkers in saliva has been a field of recent research interest.

AIM : The present aims to assess the changes in exoglycosidases and protein-bound sialic acid in blood and saliva, and carbohydrate deficient transferrin in the blood of alcohol-dependent males compared to healthy controls; and to assess the correlation between blood and saliva with respect to exoglycosidases and protein-bound sialic acid.

MATERIALS AND METHODS : The study included sixty four alcohol-dependent males and sixty four healthy controls as subjects. Blood and whole saliva samples were collected. The activities of β-hexosaminidase (HEX), α-fucosidase (FUC), α-mannosidase (MAN) and β-galactosidase (GAL), and level of PBSA were assayed in serum and saliva. The level of CDT was assayed in serum.

RESULTS : The activities of exoglycosidases and level of PBSA in serum and saliva, and CDT % in serum were significantly higher in alcohol-dependent males in comparison to controls. There was significant correlation between serum and saliva with respect to the activities of exoglycosidases and level of PBSA in alcohol-dependent males.

CONCLUSION : Exoglycosidases and protein-bound sialic acid serve as alcohol biomarkers in addition to the well-established CDT. Saliva could be a noninvasive tool for diagnosis of alcohol dependence syndrome.

KEYWORDS : Alcohol Dependence, Carbohydrate Deficient Transferrin, Exoglycosidases, Sialic acid, , Saliva

INTRODUCTION

Alcoholism is a primary illness or disorder characterized by some loss of control over drinking with habituation or addiction to the drug alcohol, causing interference in major life functions. It is a cyclic presence of tolerance, withdrawal and excessive alcohol use; the drinker's inability to control such compulsive drinking, despite awareness of its harm to his or her health, indicates the person might be an alcoholic. Alcohol dependence syndrome is a disabling disorder. It is characterized by compulsive and uncontrolled consumption of alcohol despite its negative effects on the drinker's health, relationships and social standing (1). Chronic alcohol consumption causes toxic effects on the body with involvement of multiple molecular phenomena and metabolic pathways. Consequences of alcohol toxicity include hypoxia, hypoglycemia, formation of molecular adducts and generation of free radicals. These consequences are further responsible for the clinical manifestations of alcoholism (2,3).

Identification of alcoholics especially in early stages of alcohol abuse is crucial in preventing adverse health effects and social consequences. Many biochemical parameters in blood and urine have been proposed as biomarkers of alcoholism. Major biomarkers include transaminases, gamma-glutamyl transferase (GGT) and carbohydrate-deficient transferrin (4,5). Recent studies have also analyzed exoglycosidases and sialic acid as markers of alcohol dependence (4-6).

Saliva as a diagnostic tool has received attention in recent years. Collection of whole saliva has distinct advantages of non invasiveness of its collection, non necessity of skilled technicians, least compliance problems and suitability for repeated sampling (7,8). There is paucity of studies on salivary biomarkers of diseases in general and salivary alcohol biomarkers in particular. Few studies have reported altered levels of enzymes, glycoconjugates and oxidative stress indices in saliva in alcoholics (6,9-11). In the present study, we made an attempt to assess the status of glycoconjugates and exoglycosidases in blood and saliva of alcohol-dependent males.

MATERIALS AND METHODS

Study Design and Duration :

The present cross sectional study was carried out in the Medical College Hospital for a duration of two years from March 2016 to February 2018. The protocol was approved by the Institutional Ethics Committee.

Source of Data :

The study subjects were sixty four males with Alcohol dependence syndrome admitted to Deaddiction Centre for alcohol deaddiction treatment. They were diagnosed of alcohol dependence by the treating psychiatrist, based on The ICD-10 (the 10th revision of the International Statistical Classification of Diseases) Classification of Mental and Behavioural Disorders : Diagnostic Criteria for research of WHO (12). Sixty four age- matched, apparently healthy, non-alcoholic, non smoker male volunteers served as controls. The age group of the subjects ranged from 18 to 65 years. Individuals with use of substances were excluded from the control group. Individuals with non alcoholic liver disease, kidney disease, cancer, inflammatory diseases, infections and other systemic diseases were excluded from both the study groups. Voluntary informed consent was obtained from all the study subjects.

Sample Collection:

Five ml. blood was collected between 9 -11 AM in plain vacutainers taking aseptic precautions and centrifuged to separate serum and cells. Unstimulated whole saliva sample was collected between 9-11 AM. For the saliva collection, the subjects were asked to rinse the mouth thoroughly to remove any food debris and then after ten minutes, were asked to spit into sterile plastic containers, avoiding forcible spitting (13). The saliva sample was centrifuged at 3000 rpm for 15 minutes and supernatant collected was taken for the assays.

Assays :

Carbohydrate-Deficient Transferrin in serum was determined by improved high performance liquid chromatography method (14). Transferrin was iron-saturated by mixing the serum with ferric nitrilotriacetic acid, and lipoproteins were precipitated with dextran sulfate and calcium chloride. Separation of glycoforms was performed on a SOURCE 15Q anion-exchange column using salt gradient elution. Quantification relied on selective absorbance of the iron-transferrin complex at 470 nm. The relative amount of each glycoform was calculated as a percentage of the area under the curve, using baseline integration.

Activities of β-hexosaminidase (HEX), α-fucosidase (FUC), α-

mannosidase (MAN) and β-galactosidase (GAL) in saliva and serum were determined by the method of Marciniak et al (15). The substrates used in the photometric method were, p-Nitrophenyl-N-acetyl-ß-D-glucosaminide for determination of HEX, p-Nitrophenyl-α-D-fucopyranoside for FUC, p-Nitrophenyl- α-D-mannopyranoside for MAN and p-Nitrophenyl-β-D-galactopyranoside for GAL (15). Level of protein-bound sialic acid (PBSA) in saliva and serum was estimated by the photometric method of Yao et al. in which proteins were precipitated, and sialic acid content in the precipitate was assayed based on its reaction with acidic ninhydrin (16).

Statistical Analysis :

The significance of difference of the values between the groups was evaluated by Student's t test. The correlation between serum and saliva with respect to the biochemical parameters was assessed by Karl Pearson's Correlation analysis.

RESULTS

The results of our study are presented in tables 1 to 3.

The CDT % was significantly higher in the serum of alcohol-dependent males (ADM) when compared to healthy controls. The activities of the exoglycosidases HEX, MAN, FUC and GAL in serum of ADM were significantly higher than those of controls. These activities in saliva were significantly higher in ADM when compared to controls. The level of PBSA in serum and saliva in ADM were significantly higher than corresponding levels in controls (Table 2).

The correlation analysis revealed significant correlation between saliva and serum with respect to HEX, MAN, GAL and PBSA in alcohol-dependent males. However, there was no significant correlation of FUC between serum and saliva (Table 3).

Table 1: Demographic Details of the Study Population. Values are expressed in Mean±SD.

	Controls (n=64)	Alcohol-Dependent (n=64)
Age in years	31.15 ± 9.2 (19-55)	36.9 ± 9.6 (21 - 60)
Alcohol consumption in years	---------- --	15.1 ± 7.9 (0.5 - 30)
Daily use of alcohol in years	---------- ---------	6.2 ± 5.7 (0.04 - 25)
Amount of alcohol consumption per day (grams)	---------- ----------	377.5 ± 161.5 (180 - 720)

Table 2. Levels /Activities of Biochemical Parameters in Controls and Alcohol-Dependent Males (Values are mean ± SD of number of subjects indicated in parantheses)

	Controls (n=64)	Alcohol Dependence Syndrome (n=64)	Percentage Difference (when compared to controls)	Significance of the results (P value and level of significance)
CDT ,% , Serum	0.598 ± 0.12	8.2 ± 0.34	+ 92.7 %	0.000; HS
HEX, Serum (pKat/ml)	306.16 ± 24.7	829.3 ± 27.7	+ 268.9 %	0.000; HS
HEX,Saliva (pKat/ml)	183.5 ± 23.2	543.2 ± 17.2	+ 196 %	0.000; HS
MAN,Serum (pKat/ml)	100.16 ± 14.7	346.3 ± 26.5	+ 245.7 %	0.000; HS
MAN,Saliva (pKat/ml)	83.8 ± 9.4	187.3 ± 16.6	+ 123.5 %	0.000; HS
FUC,Serum (pKat/ml)	204.6 ± 16.9	623.3 ± 23.7	+ 204.6 %	0.000; HS
FUC, Saliva (pKat/ml)	27.8 ± 7.7	134.6± 12.1	+ 384.2 %	0.000; HS
GAL, Serum (pKat/ml)	62.4 ± 9.7	98.5 ± 11.1	+ 57.8 %	0.000; HS
GAL, Saliva (pKat/ml)	11.35 ± 1.98	86.3 ±10.4	+ 660.3 %	0.000; HS
PBSA,Serum (mg/dl)	52.8 ± 8.1	83.4 ± 22	+ 57.9 %	0.000; HS
PBSA,Saliva (mg/dl)	2.62 ± 0.89	4.48 ± 1.2	+ 70. 9 %	0.000; HS

HS = Highly Significant

Table 3. Correlation of Saliva with Serum w.r.t. Biochemical Parameters in Alcohol Dependent Males

Parameter	R Value	P Value and Significance
HEX, Saliva-Serum	0.806	0.000; HS
MAN, Saliva-Serum	0.920	0.000; HS
FUC , Saliva-Serum	0.152	0.229; NS
GAL , Saliva-Serum	0.942	0.000; HS
PBSA , Saliva-Serum	0.836	0.000; HS

HS = Highly Significant; NS = Not Significant

DISCUSSION

The present study attempted to analyze the changes in carbohydrate deficient transferrin, exoglycosidases and sialic acid in alcohol-dependent males. We observed significant increase in all these parameters, and also a significant correlation between blood and saliva with respect to the alcohol biomarkers.

Elevated level of CDT in serum is used as marker of excessive and chronic consumption of alcohol. Chronic alcoholism leads to increased percentage of carbohydrate deficient transferrin, with a change in glycoforms pattern by increasing the relative amounts of disialo and asialotransferrin at the expense of tetrasialotransferrin. The present study demonstrated significantly increased blood levels of CDT in alcohol dependent males compared to controls. The study findings are consistent with the previous studies (17-19). The inhibition of sialyltransferase and induction of plasma sialidase could be involved in elevation of CDT levels in chronic alcohol abuse (17-19).

The present study demonstrated that significant elevation of salivary and blood protein bound sialic acid in alcohol dependent males compared to controls. The increase of protein bound sialic acid has been reported in several previous studies, consistent with this study (20-23). The mechanism of elevation of sialic acid in alcohol dependents is not known. It is suggested that acute ethanol ingestion alters the micro heterogeneity pattern of transferrin as a result the changes in sialic acid content (23). The association between alcohol induced desialylation of transferrin and other glycoprotein's increased the levels of sialic acid. Animal studies suggest that acute ethanol administration in animal models impair the final steps of hepatic glycoprotein secretion after the accumulation of the terminal sugars mainly in the Golgi complex. Ethanol interferes with the flow of membrane components from the Golgi apparatus to the surface of the plasma membrane. Sillanaukee et al. reported that the alcohol intake decreases the activities of sialyltransferases in Golgi and increases the activities of sialidase in both cytosol and plasma membranes, which was suggested to be the cause of increased sialic acid in chronic alcoholics (23). Acetaldehyde, the intermediate of ethanol oxidation is an important factor in ethanol-induced impairment of hepatic glycoprotein formation and secretion. We also observed a significant correlation between saliva and serum with respect to the protein-bound sialic acid levels in alcohol-dependent males.

Exoglycosidases are widely found in the tissues of humans. These act on the glycosidic bonds and release particular monosaccharides from non-reducing termini of oligosaccharides and the sugar chains of glycoproteins and glycolipids. They include alpha-fucosidase (FUC), alpha-mannosidse (MAN), beta-glucuronidase (GLU), beta-galactosidase (GAL), beta-hexosaminidase A (HEX A), beta-hexosaminidase B (HEX B). The exoglycosidases which move to the cell surface, and get secreted into the body fluids indicate the status of glyconconjuates in cells. In various diseases, increased glycations, aberrant glycations and increased catabolism cause increased levels of exoglycosidases in body fluids (24). Increases in the activity of exoglycosidases in body fluids could be the sensitive markers for preliminary screening of many diseases, including detection and monitoring of alcohol abuse, infections and cancers (24).

In the present study, we observed significant elevation in the activities of HEX, FUC, GAL, MAN in serum and saliva of alcohol dependent subjects. There was significant correlation between serum and saliva with respect to the activity of HEX, GAL, MAN but, not FUC. Waszkiewicz et al. observed increased activities of salivary FUC, GAL, GLU and MAN in alcohol-dependent individuals in comparison to healthy controls (6). The authors suggested that degradation of the oligosaccharide chains of glycoconjugates through the release of glucuronic acid from glycosaminoglycans and mannose residues from

N-linked glycoproteins, to be the most important changes in glycosylation patterns in the saliva of alcohol-dependent subjects. Activity of salivary exoglycosidases remained high in saliva even after fifty days of alcohol abstinence (25). Activity of HEX in serum has been proposed as a marker of heavy drinking (26). In the present study, highest increase in activity was seen with galactosidase in saliva (7.6 fold), followed by fucosidase in saliva (4.8 fold) in alcoholics.

CONCLUSIONS

The present study demonstrated significant changes in the glycoconjugates and exoglycosidases as an effect of chronic alcohol consumption. The biochemical parameters of blood and saliva serve as sensitive alcohol biomarkers. Saliva could be a non-invasive tool supplementary to blood in the diagnosis of alcoholism and its complications. Further studies assessing the sensitivity, specificity and diagnostic utility of salivary alcohol biomarkers would go a longway in validating saliva as a very useful diagnostic tool.

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