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Effect of cigarette smoking on serum α-L-fucose and its related parameters

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Abstract

Background and objective: Cigarette smoke is a leading preventable cause of many diseases and death, because it contains a complex mixture of chemical compounds. Few studies about the relation between cigarette smoking and glycoconjugate were found in literature, while no study was observed regarding passive smokers. Thus this relation is obscure till now. Therefore this work was designed to study the effect of cigarette smoking on serum glycoconjugates levels.

Methods: One hundred twenty one completely healthy males from different areas of Duhok governorate were included in this study. Their age was ranged between 20 and 50 years. They were classified according to the number and duration of cigarette smoking into: heavy smokers (31), moderate smokers (26), passive smokers (30) and non-smokers (control). Fasting blood was withdrawn from all groups and used for determination of serum fucose and its related parameters.

Results: The results showed that there was a significant decrease in serum total protein in heavy smoker groups when compared to non-smokers. The levels of serum total fucose and protein bound fucose significantly increased in smoker groups, while the results indicated that there was a significant increase in serum lipid associated fucose levels in heavy and passive smoker groups comparing to non-smoker group. A significant decrease in serum protein bound hexoses was observed in both heavy and moderate smokers. The result also showed that serum total calcium concentration, Ca/TP and Mg/TP ratios significantly decreased in all smoker groups.Regarding to serum Manganese (Mn) and Mn/ TP ratio, their values increased significantly in case of passive smokers only.

Conclusion: The results revealed that cigarette smoking had significant effects on glycoconjugates status (expressed in serum fucose and its related parameters).

Keywords: Cigarette smoking, Fucose, serum elements, glycoconjugate.

Introduction

Cigarette smoking is one of the most prevalent social habits practiced worldwide today and is a leading preventable cause of death and disability[1]. Cigarette smoke is a complex mixture of over 4800 identified constituents that include high concentrations of free radicals, reactive oxygen and nitrogen species, reactive aldehydes, and diverse metals[2-5]. Cigarette smoking has been implicated as a significant risk factor for the establishment and progression of several diseases including emphysema, chronic bronchitis, cardiovascular diseases, dyslipidemia and cancer[6,7]. It was documented that passive smoking is associated with a 25% increase in the risk of coronary heart disease among non-smokers; and an increase in the risk of stroke. Even brief exposure to passive smoking (e.g. for as little as 30 minutes) can affect the cardiovascular system of non-smokers. Non-smokers living with smokers have about a 25% increase in risk of death from heart attack and are also more likely to suffer a stroke[8,9]. Glycoconjugates are compounds that result from the covalent linkage of carbohydrate molecules to both proteins and

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lipids. Glycoprotein and glycolipid are the most abundant glycoconjugates found in mammalian cells. They found predominantly on the outer wall of virtually every organism occupying space between these cells (extracellular matrix). They found also in secreted fluids[10-12].Glycoproteins have multiple functions and are found as enzymes, hormones, blood group substances and as constituents of extracellular membranes. They contribute a major source to the structure of the matrix[13]. It has been published that serum glycoconjugates and their components are increased during many diseases[14,15]. α-L-Fucose is an important component of glycoproteins and glycolipids. It is a methyl pentose sugar derivative similar to galactose[16,17]. α-L-Fucose plays a big role in a variety of physiological events such as protein stabilization and function, embryologic development, reproduction, blood group recognition, immune modulation, memory and learning[18,19]. Among the nine sugars present in the structure of glycoprotein, fucose can be used reliably and conveniently for the investigations of glycoprotein synthesis and secretion[20]. Fucose studies have shown that serum fucose level increased in many disease, including cancers and their spread, rheumatoid arthritis, diabetes, Leukocyte adhesion type II, and cystic fibrosis, therefore, it can be used as a biomarker in these pathological conditions[11,21]. In 1989, Rogers *et al*, investigated that about two weeks exposure of rats to cigarette smoke significantly increased the secretion of fucose-containing glycoconjugates above normal in an in situ preparation of larynx and trachea[22]. To the best of our searching, a few or nearly no study about the effect of cigarette smoking on glycoconjugate status (expressed in serum fucose level and its related parameters) was found in literature, while no study about passive smokers was observed. Therefore this work was directed to study the effect of cigarette smoking on serum fucose level and its related parameters, thus, one can evaluated the effect of cigarette smoking on glycoconjugates.

Methods

Subjects and samples collection

One hundred-twenty one completely healthy males from different areas of Duhok City were included in this study. They were free of diabetes mellitus, hypertension, cardiovascular, and immune diseases and they have received no medications (by history taking) .There age was ranged between (20-50) years. They were classified according to the number of cigarette sticks smoked per day and the duration of smoking, into; heavy smokers (31) who used to smoke (≥20) sticks per day for ≥ 10 years, moderate smokers (26) used to smoke (10-19) sticks per day for (4-7) years, passive smokers (30) who are non-smokers, but living with smokers[23], and finally non-smokers (control) (34) who are non-smokers and living with non-smokers. About (10) ml of blood was withdrawn from each volunteer, and then it was collected in a container with no anticoagulant. The blood sample was placed at room temperature for (10-15) minutes. The serum was then being separated by centrifuge at (3000 rpm) for (10) minutes. The sera was stored at (-20C^{o}) till analysis.

Methodology

1- Serum TP was determind by Biuret method described by Gornall et al, using a special kit[24,25].

2- Serum TF level was estimated using Dische & Shettles method[26-28]. The method depends on a direct reaction of concentrated sulphuric acid with sample components. The reactants were combined together with cysteine and the color product was measured at (396) nm, and (430) nm.

3- Serum PBF level was determind using Dische & Shettles method[14,26-28]. Protein in the serum was precipitated. The precipitate was resuspended in NaOH to resolublizethe proteins. A color product was formed when fucose (in the solution), in

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strong acid medium, combined with color developer (Cysteine hydrochloride). The color intensity was measured at (396) nm and at (430)nm.

4- Serum LAF level was determined depending on Katopodismethod[27,29,30]. Serum lipid was extracted completely by a mixture of v/v chloroform and methanol (2:1). The protein will be precipitated by phosphotungestic acid, and fucose can be determined by Dische & Shettles method as mentioned in point one.

5- Serum PBHex level was estimated depending on orcinol reaction[14,27,30]. The hexose moieties of protein-carbohydrate conjugate were precipitated by 95% ethanol at room temperature and determined by the Orcinol reaction. The absorbance was measured at 520nm.

6- Serum TCa, Mg and Mn was determined by atomic absorption spectropy.

7- The ratio of each of the above parameters to TP was calculated.

Statistical analysis:

All data were expressed as means ± standard deviation (±SD). The statistical analysis was carried out using the known statistically software (SAS version 9.00). Data analysis was made using one-way analysis of variance (ANOVA).Comparison between groups was carried out using Student's *t* test.The result were considered statistically significant (S) if the (P≤ 0.01).

Results

Table 1 and Figure 1 show the mean and standard deviation (±SD) of serum total protein (TP), total fucose (TF), free fucose (FF), protein bound fucose (PBF), lipid associated fucose (LAF), and protein bound hexose (PBH) in heavy, moderate and passive smokers and non-smoker group. The results showed that there was a non-significant difference in serum TP levels between moderate and passive smoker groups compared to non-smoker groups, while this parameter decreased significantly (P≤0.01) in serum of heavy smoker groups when compared to non-smoker. The results of serum TF indicated indicated that there was a significant increase (P≤0.01) in its level in heavy, moderate and passive smoker groups (in the order of heavy> moderate> passive smoker) comparing to non-smoker group. The results of serum PBF levels showed that there was a significant increase (P≤0.01) in this parameter in smoker groups in general when compared to non-smoker, while non-significant changes were seen among smoker groups; heavy, moderate and passive smokers. The results showed that there was a significant increase (P≤0.001) in LAF levels in the serum of heavy and passive smoker groups comparing to non-smoker group. A significant decrease (P≤0.01) in serum PBH was observed in both heavy and moderate smokers comparing to non-smokers. A significant increase (P≤0.01) in serum FF was observed in smokers. Non-significant change in this parameters, was found between heavy and moderate smokers (both were higher than passive smokers). The serum values of TF/TP, PBF/TP, LAF/TP, FF/TP and PBH/TP ratios in heavy, moderate, and passive smokers and non-smoker group are shown in Table 2 and Figure 2. Serum TF/TP ratio levels increased significantly (P≤0.01) in smokers in general comparing to non-smokers, the highest level was observed in heavy smokers. Regarding to serum PBF/TP ratio, generally, smoker groups had significantly (P≤0.001) higher values than non-smoker. The results showed that the ratio of serum LAF/TP was higher significantly (P≤0.001) in smokers than in non-smokers. Regarding to the results of serum FF/TP ratio, smoker groups generally had significantly (P≤0.01) higher values comparing to non-smoker groups. Both heavy and moderate had significantly higher levels than passive smokers. Serum PBH/TP ratio was significantly lower (P≤0.01) in heavy and moderate smokers comparing to non-smokers, while no change was observed in passive smokers comparing to non-smokers. Table 3 and Figure 3 represent the Ca, Mg, Mn,

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Ca/TP, Mg/TP, and Mn/TP levels for heavy, moderate and passive smokers and non-smoker group. The result showed that serum calcium concentration and its ratio to total protein (Ca/TP) decreased significantly (P≤0.01) in smoker groups compared with non-smokers. The lowest value for both Ca and Ca/TP levels was seen in heavy smokers. In case of Mg concentration, among the smokers, only moderate smokers had a significant decrease (P≤0.01) in serum Mg concentration comparing with non-smokers, while the values of serum Mg/TP ratio significantly decreased (P≤0.01) in all the smoker groups compared with non-smoker group. Regarding to serum Mn and Mn/TP ratio, their values increased significantly in case of passive smokers only, while non-significance changes were seen in case of heavy and moderate smokers comparing with non-smoker group.

Table 1: The mean, standard deviation (±SD) of serum TP, TF, PBF, LAF, FF and PBH in heavy, moderate and passive smokers and non-smoker groups

Parameters	*H	**M	***P	****N
TP(g/dl)	6.925 ± 0.603^{b}	7.0483 ± 1.039^{ab}	7.0065 ± 0.705^{ab}	7.514 ± 0.352^{a}
TF(mg/dl)	31.719 ± 2.501^{a}	30.355 ± 2.001^{a}	22.103 ± 2.098^{b}	16.341 ± 0.693^{c}
PBF(mg/dl)	6.683 ± 1.237^{b}	6.967 ± 1.412^{b}	7.384 ± 0.728^{b}	9.606 ± 0.893^{a}
LAF(mg/dl)	4.504 ± 1.402^{ab}	3.747 ± 1.883^{bc}	4.874 ± 1.296^{a}	2.985 ± 0.944^{c}
FF(mg/dl)	20.531 ± 4.806^{a}	19.641 ± 1.407^{a}	9.845 ± 0.456^{b}	3.749 ± 1.164^{c}
PBH(mg/dl)	92.343 ± 8.398^{b}	95.019 ± 10.722^{b}	114.260 ± 15.359^{a}	121.384 ± 11.407^{a}

*H: heavy smoker, **M: moderate smoker, ***P: passive smoker, ****N: non-smoker.
Different letters in the same row refer to significant changes, while similar letters refer to non significant changes.

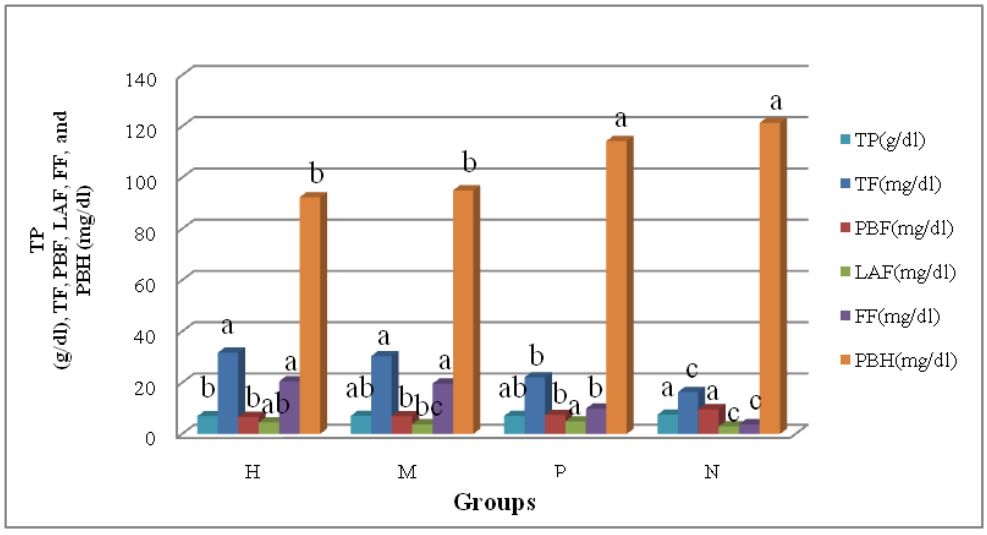

Figure 1: Serum TP, TF, PBF, LAF, FFand PBH concentrations in heavy, moderate and passivesmokersand non-smoker groups.

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Table 2: The mean, standard deviation (±SD) of serum TF/TP, PBF/TP, LAF/TP, FF/TP and PBH/TP values in heavy, moderate and passive smokers and non-smoker groups.

Parameter	H	M	P	N
TF/TP	4.637 ± 0.717[a]	2.887 ± 0.594[b]	3.140 ± 0.109[b]	2.177 ± 0.033[c]
PBF/TP	0.958 ± 0.107[c]	0.987 ± 0.074[c]	1.049 ± 0.037[b]	1.276 ± 0.062[a]
LAF/TP	0.639 ± 0.146[a]	0.511 ± 0.179[b]	0.682 ± 0.121[a]	0.393 ± 0.107[c]
FF/TP	3.040 ± 0.943[a]	2.887 ± 0.594[a]	1.409 ± 0.145[b]	0.508 ± 0.173[c]
PBH/TP	13.508 ± 2.223[b]	13.636 ± 0.643[b]	16.169 ± 0.772[a]	16.124 ± 0.865[a]

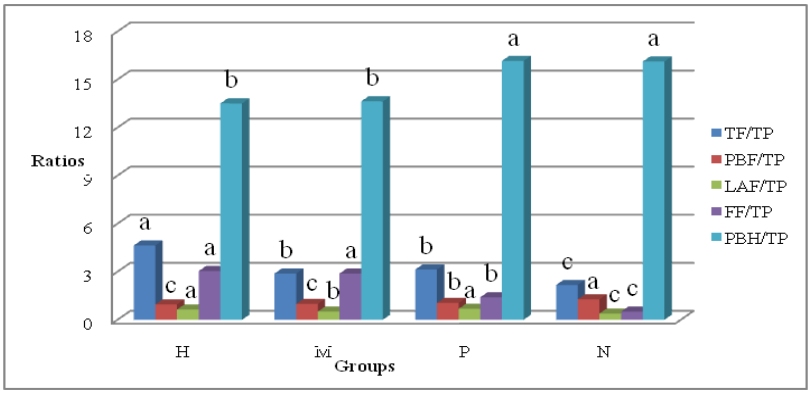

Figure 2: Serum TF/TP, PBF/TP, LAF/TP, FF/TP and PBH/TP ratios in heavy, moderate and passive smokers and non-smoker groups.

Table 3: The mean, standard deviation (±SD) of serum Ca, Ca/TP, Mg, Mg/TP, Mn and Mn/TP in heavy, moderate and passive smokers and non-smoker groups.

Parameter	H	M	P	N
Ca(mg/dl)	7.652 ± 0.533[c]	8.374 ± 0.591[b]	8.285 ± 0.564[b]	9.532 ± 1.015[a]
Ca/TP	1.107 ± 0.026[c]	1.209 ± 0.109[b]	1.180 ± 0.056[b]	1.265 ± 0.078[a]
Mg(mg/dl)	1.285 ± 0.073[ab]	1.337 ± 0.156[a]	1.249 ± 0.099[b]	1.250 ± 0.092[b]
Mg/TP	0.188 ± 0.025[ab]	0.192 ± 0.007[a]	0.178 ± 0.008[b]	0.166 ± 0.005[c]
Mn(µg/dl)	29.655 ± 4.394[b]	28.580 ± 4.911[b]	37.380 ± 5.618[a]	30.640 ± 6.280[b]
Mn/TP	4.342 ± 0.956[b]	4.097 ± 0.647[b]	5.319 ± 0.770[a]	4.068 ± 0.653[b]

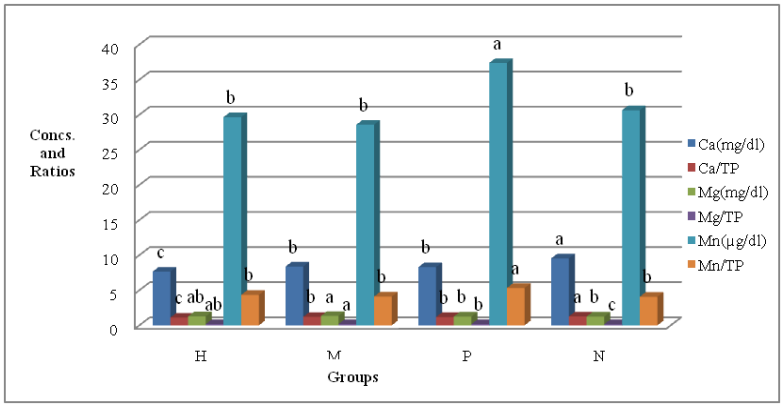

Figure 3: Serum TCa, TCa/TP, Mg, Mg/TP, Mn and Mn/TP for heavy, moderate and passive smokers and non-smoker groups.

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Discussion

In the present study, only male subjects was chosen, because serum glycoproteins may be affected by hormonal changes, which usually occur in female subjects (due to menstrual cycle, pregnancy or lactation), this is in addition to that female smokers in Kurdistan region are rare. The result of the present study showed that serum total protein decreased significantly in heavy smoker compared to non-smoker group, with no change in moderate and passive smokers. The decrease in serum total protein in heavy smokers, may be due to the presence of several toxic compounds in smoke which may affect cerebral and hepatic protein metabolism[31]. Serum TF and FF in this study was significantly elevated among smoker groups. The increase in these parameters in sera of smoker groups may be due to tissue glycoprotein degradation as a result of the effect of the toxic compounds present in tobacco and cigarette smoke. Serum LAF levels in heavy and passive smokers significantly increased. This may be due to increase in serum lipids as a result of cigarette smoke (serum lipid contains many glycolipids). In case of serum PBF level, the results showed that it decreased significantly among smoker groups when compared to non-smoker group. This may be due to the degradation of serum glycoproteins by the toxic substances, present in cigarette smoke. To the best of searching, there was no related study about the effect of cigarette smoking on serum fucose and its related parameters. In this study, it was observed that TF, LAF and FF significantly increased in smoker groups. There is indirect evidence which indicate that, increased glycoprotein components levels are offset by increased fucosidase activity which is responsible for breaking down tissue and serum glycoproteins [31].It is an important enzyme for fucose catabolism which is responsible for the altered fucosylation status of glycoproteins [33]. In the present study the activity of fucosidase may be altered by tobacco and smoke chemical components. Depending on the present results, serum fucose and its related parameters may be used as indicator for the extent of damages caused by toxic substances present in cigarette smoke. The increase in serum TF/TP, FF/TP and LAF/TP appears to be a characteristic feature of liver diseases (in this study, this increase may be due to the effect of cigarette smoke on liver cells). The decrease in serum ratio of PBF/TP, and PBH/TP is due to that, serum PBF and PBH is more affected by cigarette smoke than serum TP. It is also possible that the increase and decrease in these parameters may be return to the change in glycosidases and fucosidase activities as a result of cigarette smoking. The results of serum calcium and its ratio to total protein Ca/TP showed a significant reduction in these two parameters in smoking status. There is a relation between Ca^{+2} ion concentrations and glycoconjugates in the cell. It has been published that Ca^{+2} ions have importance roles in both; the secretion of glycoconjugates from the cells, and the binding of lectin to glycan moieties of glycoconjugates (cell-cell adhesion and cell-environment adhesion)[34]. Thus, Ca^{+2} ions are essential for the performance of glycoconjugate transport and functions, therefore, Ca^{+2} ion concentration was followed in the present study, to evaluate the effect of smoking on serum Ca^{+2} ion which is related to glycoconjugates. Lower serum calcium levels in smokers may be a pathway by which smoking affects the rate of bone loss. It may be also due to that, smoking is associated with the reduction in bone density, calcium absorption and vitamin D_3 levels[35]. Cigarette smoking has widespread effects on the body that may decrease calcium absorption and independently influence bone metabolism[36]. There was a significant increase in serum Mg level in moderate smokers when compared to non-smokers group. In case of Mg/TP ratio, there was a significant increase in the values when compared smoker groups with non-smokers group.

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Cigarette smoking may cause a breakdown or destruction of body cells, so intracellular magnesium and other electrolytes moves into circulation. Since magnesium is excreted by kidney; therefore, any damage to kidney, may cause an improper kidney work, thus, increase in serum magnesium levels. The results of this study can predict that serum manganese and its ratio to total protein may be used as an indicator for the effect of cigarette smoke on passive smokers.

Conclusion

From the result of the present study, one can conclude that cigarette smoking had significant effects on serum glycoconjugates and their related parameters in both smokers and passive smokers.

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