ORIGINAL ARTICLE
ASSOCIATION OF SURFACANT PROTEIN-D WITH OBESITY

Shireen Jawed, Nighat Mannan*, Masood Anwar Qureshi**
Department, Physiology, University Medical and Dental College Faisalabad, University of Faisalabad, *Bahria University Medical and Dental College, Karachi, **Institute of Basic Medical Sciences, Dow University of Health Sciences, Karachi-Pakistan

Background: Obesity is associated with inflammatory diseases and obese individual’s poses high risk for infections. Surfactant protein D (SP-D) is an important regulator of immunity and inflammation. Latest studies have suggested that it is also involved in lipid homeostasis and obese subjects have decrease concentration of SP-D as compared to normal weight peoples. The aim of the current study was to elucidate the relationship among serum SP-D and BMI. Method: This cross sectional study was performed at Dow University of health sciences (DUHS), Karachi. We analysed 90 obese and non-obese subjects for serum SP-D concentration. SP-D was estimated by ELISA. Data was analysed by SPSS 16. Mean SP-D level and demographical variables between the groups were compared by t test, Associations of SP-D with BMI investigated by regression analysis. Results: obese subjects have significant lower levels of Serum SP-D than non-obese and negatively associated with BMI in both genders (p=0.000). Conclusion: This study concluded that obese subjects have lower concentration of SP-D as compare to non-obese and there is an inverse association between the SP-D and BMI.

Keywords: Surfactant Protein- D, Obesity, Total Body Mass Index (BMI), inflammatory diseases

INTRODUCTION
Obesity has become great health challenge because of the alarming rise in its prevalence globally. It is increased from 29.8–38.0% in women and from 28.8–36.9% in men between 1980–2013.1 Asia, Middle East and Pacific Islands all at high risk.2 According to WHO prevalence of obesity in Asia is 10–30%.3 In urban areas of Pakistan Prevalence is 22–40%.3 BMI and WHR are two important parameters for assessing the level of obesity.4 According to international classification of obesity by WHO BMI >25 kg/m^2 are considered as obese in Asian population.5 Metabolic diseases such as type 2 diabetes mellitus, hypertension and dyslipidaemia are highly associated with obesity.6 Along with these co-morbidities obese individuals have greater risk for getting infections than normal weight individuals. It is an important predictor of an increased incidence and severity of various types of infections including respiratory tract infections probably because of defects in immune system.7 Obesity is associated with inflammation with abnormally increased production of cytokine, elevated acute-phase reactants, and responsible for inflammatory signalling pathways. Inflammatory process triggered in adipocytes.8 Attention has been focused towards the consequences of obesity like metabolic disorders, cardiovascular diseases,9 however, effect of obesity on immune functions has been poorly characterized. Surfactant protein- D (SP-D) is calcium dependent, collagen-containing C-type collectin predominantly produced in respiratory tract,10 but is also found in gastrointestinal, tract bile ducts and extra pulmonary tissues.11 SP-D is the key components of innate immunity in respiratory system. It acts as a first line of defence against the inhaled microorganisms and antigens.12 SP-D alters the secretion of inflammatory baseline meaditers post-prandial cytokine synthesis which affect fat metabolism. Binding of SP-D with free fatty acids affects its cellular uptake and catabolism in obese individuals which may results in its deficiency in these individuals. Recent epidemiological studies have supported the evidence that individuals having high BMI have lower concentration of SP-D which almost probably involved in pathogenesis of infections. Exact mechanism of lower levels of SP-D in obesity is still not clear but it has been postulated that there may be genetic contribution.14

We do not have enough data available for Pakistan to demonstrate the SP-D concentration in obese individuals. It is therefore imperative to find out SP-D levels in obese subjects in Pakistan. The objective of this study was find out association of obesity with surfactant protein-D.

MATERIAL AND METHODS
This cross sectional study was conducted during September 2011 to April 2012 at Dow university of Health Sciences (DUHS), Karachi following the approval from the institutional review Board (IRB) of the DUHS. Non probability purposive sampling was used to select study population 90 subjects of 30–60 years who met the selection criteria were recruited by from employees of DUHS in the study. Information’s about their demographic details, physical activity, dietary habits, smoking, and socioeconomic status, medical history, was recorded

http://www.jamc.ayubmed.edu.pk
on pre-designed pro forma. Anthropometric measurements were taken by standard protocols. WHO guide lines on BMI cut off points for Asians were used to determine obesity. Total 90 subjects were divided into two following groups according to BMI.

Group 1 comprises of 45 obese individuals with BMI greater than 25 kg/m²

Group 2 comprises of 45 non obese individuals with BMI less than 25 kg/m²

After taking informed consent and explanation of procedure and purpose of the study, all participants underwent blood sampling, five cc of blood samples were drawn by venepuncture from all the subjects under sterile conditions. Serum separator tubes (SST) Serum separator tubes contain a gel that separates serum from the blood were used to collect blood samples for SP-D. Each sample for SP-D was allowed to clot at room temperature for half an hour and then serum was separated by centrifuging for 20 minutes. All Sera were stored in labelled Eppendorf tubes at -80 °C in Dow diagnostic research and reference lab (DDRL) until analysed. Each blood sample was analysed for SP-D.

Serum SP-D was analysed at DDRL by Enzyme Linked Immuno Assay Kit (Human SP-D ELISA KIT, DE194059101) which was commercially available from de-medi-tec laboratory Germany. The assay has sensitivity of 0.01 ng/ml.

Standards, quality controls and samples to be assayed were incubated in pre coated microtiter wells. SP-D molecule present in the sample was bound to the immobilized antibody molecules. After 120 minutes of incubation and washing biotin labelled monoclonal anti human SP-D antibody was added to the reaction mixture and incubated with previous bond SP-D molecule for 60 minutes creating an antibody-antigen-antibody "sandwich".14

After washing away any unbound reagents Streptavidin HRP conjugate was added for 60 minutes for incubation and then after again washing, the TBM substrate solution was added to react with remaining HRP conjugate to develop yellow coloured product. Acidic solution was added to stop the reaction. The absorbance of coloured product was measured which was proportional to the concentration of surfactant protein D.14 Multichannel spectrophotometer Vmax microtiter plate reader was set to 490 nm to read the absorbance.

Statistical analysis was conducted using SPSS 16. Demographic data of study population was evaluated by descriptive statistics. Continuous variables (Age, BMI, height weight and SP-D levels) were expressed as mean±SD. Differences in mean of demographic and biochemical variables between two study groups were tested statistically by using t test. Regression model was used to analyse association of dependant variable (SP-D) with independent variables (BMI). The p value <0.05 were taken as significant.

RESULTS

The subject profile shows that study population consisted of 52.6% males and 26.3% females with mean±SD the age of 42±1.03 years. Serum SP-D and Basic characteristics of two groups were compared by t test. Statistically significant differences were noted between the obese and non-obese groups with respect to mean of age, height weight, BMI and SP-D. (Table-1) Serum SP-D was significantly lower in obese subjects as compared to non-obese subjects. Serum SP-D concentration was negatively associated with BMI, analysed by regression analysis (R²=-.122, beta coefficient (β)= -7.136, standard error (SE) =1.768, p value=0.000). Figure-1 shows regression analysis between SP-D and BMI.

Figure-1 shows scatter plot indicating the association between SP-D and BMI. The fitted line indicates negative linear relationship resulting from regression between variables.

Table-I: Comparison of variables between study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Obese (n=45)</th>
<th>Non obese (n=45)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>43.94±10.15</td>
<td>41.1707±11.56</td>
<td>0.0001</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>75.12±11.00</td>
<td>60.9±9.3</td>
<td>0.0005</td>
</tr>
<tr>
<td>Height(cm)</td>
<td>1.62±0.098</td>
<td>1.65±0.09</td>
<td>0.0003</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>29.80±5.38</td>
<td>22±2.50</td>
<td>0.000</td>
</tr>
<tr>
<td>SP-D</td>
<td>78.2±43.3</td>
<td>147.3±13.4</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Statistically Significant difference at p<0.05, BMI=total body mass index, SP-D=Surfactant protein –D

DISCUSSION

Obesity is closely associated with an increased risk infections and inflammatory diseases. Evidences from the prior researches indicate that obese individuals are more likely to get various types of
infections with the consequence of serious complications than normal weight subjects. Obesity is associated with inflammation characterized by abnormal raised cytokine, elevated acute-phase reactants, and responsible for inflammatory signalling pathways. The inflammation triggered in adipocytes. Obesity has an obvious but still not well defined effect on the immune response through a variety of immune mediators, which leads to vulnerability to infections. SP-D alters the secretion of inflammatory baseline mediator’s post-prandial cytokine production which greatly affect fat metabolism. SP-D binds to free fatty acids and enhances catabolism of fatty acids and lipids. Recent epidemiological studies have reported that the individuals having BMI greater than 25 kg/m² have lower levels of SP-D. Current research was conducted to highlight the relationship between SP-D and obesity. SP-D is crucially important for immune system and its role in immunity has been well documented. It is predominantly produced and secreted by type 2 alveolar and non-ciliated airways Clara cells into lung fluids. SP-D was primarily detected in respiratory tract, several studies illustrated that it is present in all mucosal surfaces and blood. The mean levels of SP-D were compared between obese and non-obese groups. Results of current study indicate significant lower levels of SP-D in obese subjects (78.2 ng/ml) as compared to non-obese individuals (147.3 ng/ml) These finding were consistent with findings of with Zhao XM et al. who also found lower SP-D level in obese than non-obese individuals. Relationship between the SP-D and BMI was analysed by regression analysis. The results of this study reveal significant negative association between BMI and SP-D (r² = -0.122, p=0.000), β Coefficient of -7.13 demonstrates that 7.13 ng/ml of SP-D fall is associated with one unit increase in BMI. These findings are almost similar to the prospective study conducted by Sorensen GL which reported strong negative association of BMI with SP-D in both genders with β coefficient of -0.649 women −0.893 men (p=0.039 women, p=0.001 men), Indicating 6–8% reduction in SP-D levels with one unit rise in BMI. The exact mechanism of lower levels of SP-D in obesity is still ambiguous but various studies have shown that there may be genetic contribution. Gene for SP-D is SFTPD located on chromosome 10. Studies on Danish twins by Sorensens GL illustrated very strong genetic influence on serum SP-D levels. Significant genetic correlation between serum SP-D and metabolic variables have been reported by some studies. Recent study conducted by Ortega FJ also reported inverse association of lung-derived circulating SFTPD gene with obesity. Inverse association of BMI with SP-D was also reported by study conducted by Shakoori TA at Lahore Pakistan.

There is a need to evaluate the exact cause of lower levels of SP-D in obesity and to clarify the role of SP-D in infections. Further studies are required on a large scale to authenticate the negative association between SP-D and BMI.

CONCLUSION

Present study concluded that the obese individuals having BMI greater than 25 kg/m² have lower levels of SP-D than non-obese individuals but the mechanism is remains a hypothesis. New researches on broader scale are required to explore exact mechanisms involved in lower levels of SP-D in obesity.

AUTHOR’S CONTRIBUTION

SJ: Study design, data collection, statistical analysis, interpretation of results, and formulation of tables, manuscript writing and revising it critically for important intellectual content. NM: study design and supervision of the project. Revising the article critically for important intellectual content. MAQ: study design supervision of the project, reviewed and approved the manuscript

REFERENCES


Address for Correspondence:
Dr Shireen Jawed, House No: 79/C, University Town, Sarghoda Road, Faisalabad-Pakistan
Cell: +92 300 702 2067
Email: drshireenjawed@gmail.com