

# ASSOCIATION OF NORMAL WEIGHT OBESITY WITH SERUM SURFACTANT PROTEIN - D

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## ABSTRACT

**Objectives:** To find frequency of normal weight obesity in males and females and to explore its association with the serum Surfactant protein –D (SP-D). We assessed and compared infection rates among genders and tried to establish its link with normal weight obesity and SP-D.

**Material and Methods:** This cross sectional study was performed at Dow University of Health Sciences (DUHS), Karachi from 2012 to 2013, after approval from the Institutional Review Board (DUHS/DR/2011/892). It comprised of 120 participants of both genders with age ranging 30-60 years. Subjects were recruited by non-probability purposive sampling technique. Structured proforma was used to record history of infections. Waist-hip ratio (WHR) was calculated from recorded Waist and hip circumferences. Cut off points for males and females normal weight obesity were taken as 0.88 and 0.86 respectively. Surfactant protein –D (SP-D) was determined by ELISA. Statistical analysis was performed by SPSS 20. Mean of SP-D was determined and compared by Independent t- test. Percentages for normal weight were estimated. Chi square test was used for association of normal weight obesity with gender. Odd ratio was estimated using binary logistic analysis. Association between SP-D and WHR was evaluated by regression analysis.

**Results:** Out of total 120 studied subjects, 68.3% and 31.7% were males and females respectively. Females have comparatively lower SP-D levels than males ( $85.5 \pm 32.50$  Versus  $152.12 \pm 88.00$ , p value 0.001). 26.3% of females and 12.2% of males had normal weight obesity (P value 0.05). Female poses 2.27 times more risk for generalized infection than male population. (OR= 2.27, P value 0.04). WHR and SP-D are negatively associated (Beta coefficient ( $\beta$ ) of - 3.15, (P value 0.0001)

**Conclusion:** Normal weight obesity is frequently found in females than in males. Females have lower concentration of SP-D levels and high risk of infection.

**Key words:** Infections, Normal weight obesity, SP-D, Waist-Hip ratio

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## INTRODUCTION

Obesity is a huge health burden worldwide. It is a significant risk factor for diverse infections including respiratory, dermatological, UTI, wound infections and nosocomial infections due to impaired immune responses that highlight close interaction between metabolic control and immune tolerance<sup>1</sup>. Concerning debates on infection among gender, diversity was documented by previous researches. Some researchers have reported that the der-

matological and subcutaneous tissue infections commonly develop in males, whereas respiratory tract infections and urinary tract infections are present more frequently in obese females. Abscesses are found commonly in obese subjects of both genders<sup>2</sup>. Body mass index (BMI) is most widely used parameter for assessing obesity, however it is not indicator of measuring visceral adipose tissue (VAT) as it is calculated by height and weight only. BMI has less sensitivity for predicting body fat percentages that results in misclassification of subjects with excess adiposity as normal weight<sup>3</sup>. Normal weight obesity is now new emerging concept for researchers and it is used for assessing obesity by various researchers. Normal weight obesity (NWO), is a condition in which individuals are classified as normal weight by BMI, but have excess body fat<sup>4</sup>. Evidences are available showing that subjects with NWO have more risk of getting metabolic disorders and infections than BMI index obesity due to excess fat accumulation that results in disruption of lymphoid tissue integrity and alters secre-

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tion of adipo-cytokines causing immune dys-regulation<sup>5</sup>. Normal weight obesity is prevalent in up to one-third of individuals of Asian ethnicities but still it is unrecognized in various Asian countries<sup>6</sup>. NWO is not extensively studied but latest researches are now focused on it; that seem to be reliable predictor of health risk in future. Literature is emerging on its association with metabolic disorders including type 2 diabetes mellitus, dyslipidemia, hypertension and cardiovascular mortality<sup>6</sup>. Several studies have demonstrated the importance of waist circumference and WHR as a marker of VAT and emphasize to use these parameters for assessing NWO<sup>4</sup>.

SP-D is a part of collectin family of pattern recognition receptors (PRR) having antimicrobial effects<sup>7</sup>. It plays key role in innate as well as adaptive immunity against inflammation, allergy and infections<sup>8</sup>. By direct interacting with microbes and modulation of host cell responses via series of cellular receptors, it causes dampening of inflammation and innate signaling evoked by microbe-derived ligands<sup>9</sup>. It has a pivotal role in phagocytosis during aggregation and augmentation of microbes for elimination from pulmonary as well as extra pulmonary sites<sup>10</sup>. Primarily it focused on chemotaxis, opsonization, pattern recognition and binding with bacteria, yeast, helminthic parasites and viruses to promote their attachment to phagocytic cell through Fc receptors on fragment crystallization region of immunoglobulin and complement receptors to facilitate their destruction and elimination from body to prevent wide variety of infections<sup>7,11</sup>. It directly interacts with myeloid lineages to enhance clearance of microbes by macrophages<sup>10,11</sup>. Moreover, it facilitate efficiency of Neutrophil Extracellular Traps (NETs), bactericidal and fungicidal activity and neutralization of infectivity caused by these pathogens<sup>8</sup>. It also enhances nuclear and cell membrane blebbing suggesting its role in apoptosis thus prevention from cancers<sup>12</sup>. Deficiency of SP-D has been linked extensively in medical literature with a multitude of infections including, respiratory tract infections, Helminth infections, urinary tract and dermatological infections<sup>9,11</sup>. Its deficiency also leads to various types of cancers like pancreatic cancer, ovarian cancer, prostate cancer<sup>10,12</sup>. This signifies the importance of SP-D in our body and the role it plays in innate immunity. There is a huge variation in the frequency of infections among genders and also conflicting results concerning the difference in SP-D levels among the gender have been reported by several previous researches. There is need to assess status of normal weight obesity, SP-D level and infection rate in both genders separately, so in future the link between them can be clarified. In future SP-D could be used for therapeutic purpose especially in subjects with recurrent infections. Normal weight obesity is an under recognized yet widely prevalent problem in individuals of Asian descent<sup>6</sup>. This study was planned to assess frequency of new emerging concept of normal weight obesity (NWO) that seem to be reliable predictor of

health risk in future<sup>3,6</sup>. We also planned to estimate SP-D levels in males and females and to establish its association with NWO. Furthermore we assess the infections rates and predict relative risk of infections in both genders. This study highlights the importance of identifying high risk normal weight obese subjects and helps in understanding the relation of NWO with infections and SP-D.

## MATERIAL AND METHODS

A cross sectional study was carried out at Dow University of Health Sciences (DUHS), Karachi from 2012 to 2013, after approval from the Institutional Review Board (IRB) (DUHS/DR/2011/892). It comprised of 120 male and female participants. Sample Size was Calculated with prevalence (p) of obesity 13%, error (e) 5% and confidence interval (CI) of 95% by formula  $n = Z^2 \cdot p(1-p) / e^2$ . (n = sample size; Z = standard normal Z value at 95% CI = 1.96)<sup>13</sup>. Healthy Employees of DUHS with age range 30 to 60 years were included in the study. All Study participants were selected by non-probability purposive sampling technique. Informed consent from subjects was taken prior to enrollment. Study subjects were evaluated for recurrent infections on the basis of thorough medical history. Past history for respiratory and general infections including GIT, skin and urinary tract infections were taken. The frequency, duration, severity, complications of infection and use of antibiotic were inquired to evaluate recurrent infection rates. All relevant information was recorded on predesigned proforma. Subjects having three or more severe infections in one year, or the need for antibiotics for two months/year were considered as having recurrent infections<sup>14</sup>. All information was kept confidential. Smokers, subjects with known history of chronic renal diseases, end stage renal disease, cardiovascular and lung diseases and cancers that affect the SP-D levels and possible cause of infections were excluded from study to minimize the confounders. Anthropometric measurements like, hip and waist circumferences were taken by standard protocols. Waist and hip circumferences are measured in inches using measuring tape. Waist-hip ratio (WHR) was calculated from recorded waist and hip circumferences. Normal weight obesity was assessed on the basis of WHR. WHR cut off points for males and females normal weight obesity (NWO) were taken as 0.88 and 0.86 respectively<sup>15</sup>. Blood samples were taken from subjects. Serum SP-D level was determined by sandwich ELISA technique (Demedi-tec Laboratory, Germany). Statistical analysis of data was performed by SPSS-20. Mean  $\pm$  SD were used for describing quantitative variables. Categorical variables (gender, NWO infections) were presented as percentages and frequencies. Normality of data was checked by Shapiro Wilk test<sup>13</sup>. Data was normally distributed as p value was insignificant. Independent t test was applied to compare mean differences of anthropometric and SPD concentration among the gender. Infections were taken as dichotomous variables ("yes" and "no"). Binary logistic re-

gression was used to predict the risk of infection among gender and results are expressed as odd ratio (OR). Chi square was used to assess relationship of NOW with gender and infections, frequencies are also compared by Chi square test. Linear regression analysis was used to explore association between Dependent variable (SP-D) and independent variable WHR and results are expressed as beta coefficient and standard error. P-value  $\leq 0.05$  was considered as statistically significant.

## RESULTS

Data was collected from 120 male and female subjects with mean age  $45 \pm 11.65$  years. Of total subjects, 82(68.3%) were males and 38(31.7%) were females. Table 1 is describing the comparison of mean anthropometric measurements among the gender. Significant differences were noted with respect to waist and WHR, with P values 0.042 and 0.031 respectively. Table 2 is summarizing that the females have higher frequency of NWO (p value 0.04), higher rates of infections (self-reported) along with lower levels of serum SP-D (Pvalue 0.001) as compared to male population. 60.5% of the females reported the history of recurrent infections as compared to this only 43.9% males have recurrent infections table 2. Estimated OR of 2.27 shows that females have comparatively 2.27 times higher risk of infections than males (P value 0.04). Simple Linear regression analysis shows significant negative association between WHR and SP-D. Beta coefficient ( $\beta$ ) of - 3.15 indicates that one unit increase in WHR, decreases SPD by 3.15units (P value 0.0001) table3.

**Table 1: Comparison of Anthropometric Measurements among genders (N=120)**

Study Variables	Male	Female	P value
	Mean $\pm$ SD	Mean $\pm$ SD	
Hip Circumference (inches)	62.95 $\pm$ 10.90	64.86 $\pm$ 15.76	0.901
Waist Circumference (inches)	46.13 $\pm$ 10.31	52.10 $\pm$ 14.44	0.042*
WHR	0.70 $\pm$ 0.15	0.79 $\pm$ 0.15	0.031*

WHR=Waist – hip ratio, SD=Standard deviation  
Statistically Significant difference at P  $\leq$ 0.05

**Table 2: Comparison of study Parameters among gender.**

Gender	NWO N (%)	SP-D (ng/ml) Mean $\pm$ SD	Recurrent Infections	
			Yes (%)	No (%)
Male (n= 82)	10 (12.2%)	152.12 $\pm$ 88.00	36(43.9%)	46(56.1%)
Female (n= 38)	10 (26.3%)	85.51 $\pm$ 32.50	23(60.5%)	15(39.5%)
P value	0.05*	0.001*	0.04*	

Normal weight obesity (NWO) is on the basis of WHR. Surfactant protein –D (SP-D)

Infections are self-reported, Odd Ratio (OR)= 2.27

Mean are compared by T-test, frequencies are compared by X2test

P Value  $\leq$ 0.05 was considered than Statistically Significant

**Table 3: Regression analysis between SP-D and WHR.**

Independent variable	Beta Coefficients ( $\beta$ )	Standard Error	P value	
Waist Hip ratio (WHR)	-	3.15	62.59	0.0001*

Dependent Variable is Surfactant Protein –D (SP-D)

Statistically Significant difference at P  $\leq$ 0.05

## DISCUSSION

Current study highlighted the association of SP-D with normal weight obesity among the both the genders. Additionally we evaluated the infection rate among them and estimate OR to predict risk for infections in female and male population. WHR is the reliable indicator of normal weight obesity, so in this study we tried to explore relationship between SP-D and WHR and infections with respect to gender. Current study reveals that 26.3% of female population had normal weight obesity in contrast to this 12.2 % male have NWO. Furthermore, we have observed that greater percentages of females have history of infections than males. Odd ratio of 2.27 of our study favors the above findings indicating that females have 2.27 times more tendencies to have infections as compared to males, most probably because of the fact that females have greater WHR and have normal weight obesity. Our findings are justified by Dobner et al who reported the increase in overall infections with increase in BMI and obesity<sup>1</sup>. Our results are also in line with previous study by Kaspersen et al, that reported the increasing risk of infection with obesity<sup>2</sup>. It is also evident from the previous studies that the obesity is associated with decrease in SP-D levels and can leads to infections<sup>9,16</sup>. Current study also found significant decrease in the concentrations of SP-D in females than males. On contrary to our results another study conducted in Lahore did not find significant difference in SP-D levels among gender<sup>17</sup>. However, similar results to current study was reported from the neighbor country China, that demonstrated significant lower concentration of SP-D in females as compared to males<sup>18</sup>. Study in Danish population is also in agreement of current study that documented the higher SP-D concentration in males than females<sup>19</sup>. Baseline SP-D levels in Sindhi population of the current study was higher as compared to the levels reported from study conducted at Lahore among Punjabi population. SP-D concentration of male and females of current study are 152 and 85 ng/ml respectively, while study conducted in Lahore have reported 8.43 ng/ml of SP-D in control healthy smoker group of both genders, this distinction might be because of ethnical and racial differences<sup>17</sup>. Dissimilarity may be because of the fact that aforementioned study determined the levels in smokers; however our studied population was nonsmokers, also suggesting the impact of smoking on SP-D levels. Another factor for concerning the disparity in results of two Pakistani studies could be the use of two different media for determination

of SP-D levels. Current study had used serum for estimation of SP-D level while Plasma was used by study conducted at Lahore<sup>17</sup>. Evidences are available showing that serum has 20-36% greater amount of SP-D than plasma<sup>18</sup>. Moreover we have observed a negative association between the WHR and immune-regulator SP-D levels. This suggests that there is decreased immunity in subjects with increased WHR. This further supports the statement that females have more tendencies to have infections due their higher WHR and the corresponding lower SP-D levels. Obesity is the pivotal factor here; leading to a decreased level of SP-D. Previous published data favors the current results that showed negative association between SP-D and BMI indexed obesity<sup>16</sup>. The present study emphasizes the need of a better model to understand the relationship and the reasoning behind the decreased SP-D levels in subjects with increased WHR. The recognition of WHR as a standard of obesity should be enforced and further studies should be carried out on a larger scale with these parameters. Consequently, gaining a better understanding of the risk factors that are associated with normal weight obesity is an important step towards prevention, therapeutic options, appropriate management and making policies to promote healthy lifestyles<sup>20</sup>. This study adds literature concerning normal weight obesity in Pakistan, as new researchers are being exploring NWO in Asian population and trying to identify this high risk group in this region<sup>6-20</sup>. This study will be open horizon for new researches on a broader scale.

### LIMITATION

Casual association is not established due to cross sectional nature of study. Results may not be generalized to whole population because of small sample size. Study should be conducted on broader scale to evaluate mechanistic link between SP-D and normal weight obesity

### CONCLUSION

Normal weight obesity frequently found in females than males. Females have lower concentration of SP-D levels and high risk of infection might be because of higher WHR than males.

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#### **AUTHOR'S CONTRIBUTION**

Following authors have made substantial contributions to the manuscript as under

**Jawed S:** Study design, data collection, interpretation of results, writing and revising all intellectual contents of manuscript.

**Parveen N:** Data collection, statistical analysis of data, interpretation and write up of results.

**Roha:** Manuscript writing, data analysis and revising it critically.

**Altaf B:** Manuscript writing and revising final version of article .

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.