Relationship of surfactant protein-D with random blood glucose and extra pulmonary infections in type-2 diabetes mellitus

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Abstract

Objectives: To compare surfactant protein-D levels in type 2 diabetics and non-diabetics, and to explore its link with random blood glucose and extra-pulmonary infections.

Methods: The case-control study was conducted in 2012-13 at the Dow University of Health Sciences, Karachi, and comprised diabetic cases and non-diabetic controls. Extra pulmonary infections, body mass index and random blood glucose levels were noted and serum surfactant protein-D was analysed using enzynme-linked immunosorbent assay. Data was analysed using SPSS 20.

Results: Of the 120 subjects, 60(50%) each were cases and controls. Significant negative correlation was found between surfactant protein-D and infections (rs= -0.495, p=0.0001). A negative association between random blood glucose and surfactant protein-D was found, but it was not significant (p=0.15). Relative risk for extra-pulmonary infections in diabetics was 2 times higher than the controls (odds ratio: 2.10, p=0.04).

Conclusion: Serum surfactant protein-D was found to have negative association with extra-pulmonary infections.

Keywords: Extra-pulmonary infections, Hexokinase method, Random blood glucose, Surfactant protein-D.

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Introduction

Surfactant protein-D (SP-D) is a collecting molecule responsible for immune regulation at cellular level.[1] It has a critical role in innate as well as cell-mediated immunity and is intrinsically linked to metabolic homeostasis.[2] Its main function is to enhance the opsonisation, phagocytosis and aggregation processes of microbial organisms and host cells which have undergone significant damage.[3] It also plays an important role in inflammation, having both pro- and anti-inflammatory capacities.[4] SP-D is most commonly identified as a component of surfactant in the lungs, but it is not exclusive to the lungs and is significantly expressed in non-pulmonary tissues, such as skin, eye, ear, nasal compartment, epithelia of the urinary, glandular, circulatory system, gastrointestinal and reproductive tracts.[3] It is also located in the microvascular endothelium of sub-synovial and pannus tissues of joints.[3,4] Altered protein expression and variation of genetic makeup of SP-D has been shown in a number of extra-pulmonary pathologies, such as rheumatoid disease, problems of organs such as the skin, intestines and the renal system etc.[3,4] Human SP-D also appears to play distinct roles in controlling generalised infection, allergy and inflammation.[5] It combats infections by acting against pathogens through pattern recognition, opsonisation, aggregation and agglutination. It enhances phagocytosis by macrophages and neutrophils, and produces free radicals as well as exhibits fungistatic behaviour. SP-D is also involved in manipulating cytokine and chemokine profiles during inflammation generated by infection, allergen, apoptotic and necrotic cells.[3,6] It also has key role in enhancing T cell immunity. Other noteworthy functions of SP-D include speeding up the expulsion of apoptotic matter and deoxyribonucleic acid (DNA) material and aiding in the removal of allergens.[3]

Diabetes mellitus (DM) is one of the largest emerging threats to health in the 21st century.[7] It is a chronic debilitating disease causing life-long complications. It leads to generalised multiple infections, macro- and micro-vascular pathologies and, hence, can lead to increasing psychological and physiological stress.[8] Most infections are related to lung, cardiac tissue, gastrointestinal tract (GIT), renal as well as dermatological conditions like cellulitis, sepsis, bone and joint infections with potentially more serious consequences.[8,9] Infections leading to amputation of lower limbs, malignant external otitis, rhinocerebral mucormycosis and gangrenous cholecystitis are also more frequently developed in diabetic subjects.[7,10] Hyperglycaemic environment, which favours immune dysregulation, such as neutrophil dysfunction, depression of the antioxidant system, low

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production of cytokines, reduced response of T cells and humoral immunity, is the most probable mechanism of frequent infections in diabetic patients.[11] Pathogens to which diabetic patients show particular susceptibility are known to bind and agglutinated by SP-D for clearance, hence it has a role in combating infectious diseases which are more prevalent in individuals with DM.[12] These include bacteria like staphylococcus (S.) aureu, escherichia (E.) coli, klebsiella (K.) pneumonia, pseudomonas (P.) aeruginosa, and other pathogenic bacteria.[3] Enveloped viruses like human immunodeficiency virus (HIV), influenza A virus and fungi, including Candida (C.) albicans, alternaria tenuis, aspergillus (A.) fumigatus and cryptococcus neoformans.[3] SP-D can also defend against human reproductive system infections caused by chlamydia trachomatis.[3] Presence of SP-D in the female reproductive tract combats sexually-transmitted infections by interacting with HIV and potentially inhibits its infectivity of cluster of differentiation 4 (CD4) T cells directly. It also enhances the binding of HIV to immature monocyte-derived dendritic cells (iMDDCs), and facilitates HIV capture and transfer infectious HIV particles from Dendritic cells (DCs) to T cells, and, hence, it is a dual modulator of HIV infection.[13] SP-D plays a protective role against urinary tract infections (UTIs) by inhibiting the growth of uropathogenic bacteria, such as E. coli and reducing bacterial adherence to human bladder cells. It has an important role in the prevention of tubulointerstitial fibrosis, which is the common pathway to end-stage renal disease, over-expressing SP-D in human kidney suppresses the expression of monocyte chemoattractant protein-1 which is responsible for progression of this renal disease.[14] Expression of SP-D in outer layers of skin which are in direct contact with pathogens explains its role in innate immunity of skin. Additionally, the strong expression SP-D in inflammatory pathologies support its role in inflammatory diseases of skin, like psoriasis and atopic dermatitis.[3] Expression of SP-D in extra-pulmonary regions and their interaction with various pathogens highlights its generalised role in innate immunity and is evident of widespread activities. In the future, it might be useful as a means for the prevention and novel therapeutic approaches for the treatment of common infections, especially in immuno-compromised patients, such as subjects with DM. Attempts to correlate SP-D glucose intolerance and generalised infections in diabetes have been done previously, but nothing has been ascertained yet.[11,12]

The current study was planned to compare the SP-D levels among diabetic and non-diabetic individuals and to find link between SP-D and random blood glucose (RBG).

Subjects and Methods

The case-control study was conducted in 2012-3 at the Institute of Basic Medical Sciences (IBMS), Dow University of Health Sciences (DUHS), Karachi, in collaboration with the National Institute of Diabetes and Endocrinology (NIDE) and the Dow International Medical College (DIMC), DUHS, Karachi. After approval from the DUHS institutional review board, the sample size was calculated using Open-Epi calculator with prevalence (p) 13%, error (d) 5% and confidence interval (CI) 95%.[15] This study comprised of diabetic and non-diabetic subjects of 30 to 60 years (60 in each group) including both genders. The sample was raised using non-probability purposive sampling from among diagnosed diabetic subjects attending the out-patient department (OPD) of NIDE during their regular visits, and non-diabetic subjects from among the employees of DIMC. Those included were diagnosed diabetic patients aged 30-60 years without any other known systemic and endocrine diseases, and age-matched healthy subjects without diabetes or any other disease. Type 2 DM (T2DM) was defined according to the criteria recommended by the American Diabetes Association.[16] Patients having random blood glucose (RBG) $\geq$200 mg/dl on 2 or more occasions, two-hour post-glucose load (75g) plasma glucose $\geq$200 mg/dl during oral glucose tolerance test (OGTT), fasting blood glucose (FBG) $\geq$126 mg/dl or glycated haemoglobin (HbA1c) 6.5% were diagnosed as cases.[16] The healthy subjects acted as controls. Those excluded were subjects with known history of other endocrine disorders, liver diseases, malignancy, cardiopulmonary diseases as well as drug addicts and smokers. Patients on insulin therapy were also excluded.

After taking informed written consent from each participant, extra-pulmonary infections were evaluated on the basis of medical history by using pre-designed proforma. Two or more severe infections in one year, or the need for antibiotics for two months/year were considered recurrent infections.[17]

Body mass index (BMI) was calculated by recording height in centimetres and weight in kilogram by stadiometer and weighing scale (ZT-120 health scale) respectively. BMI is a ratio of weight in kilogram to squared height in meters (kg/m^2) and is a measure of body fat and obesity.[18] Under sterile conditions, 5cc blood samples were drawn by venipuncture. Blood samples for RBG were collected in sodium fluoride tube to avoid glycolysis, and serum separator tubes (SST) were used for SP-D collection. 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 laboratory (DDRRL) until analysed. Each sample was analysed for RBG and SP-D. RBG was determined by hexokinase method by automatic biochemical analyzer (Hitachi 902, Roche diagnostic) which automatically calculated the analyte concentration of each sample. The analyzer used photometric technique for glucose estimation. SP-D was analyzed using enzyme-linked immunosorbent assay (Kit: DE194059101, De-medi-tec Laboratory, Germany). The assay had sensitivity of 0.01ng/ml.

Data was analysed using SPSS 20. It was expressed as mean ± standard deviation or frequency and percentage. Normality of data was checked by Shapiro-Wilk and the Kolmogorov-Smirnov tests. After stratifying assumptions, Independent t-test was used for comparison of mean SP-D, RBG and anthropometric measurements in the study groups. Chi-squared test was used to compare categorical variables between the groups. Simple linear regression analysis was applied to analyse the association between RBG and SPD. Relation between SP-D and infection was analysed by Pearson correlation. The study population was sub-divided into subjects with infection and without infections for comparison of SP-D levels among the total population on the basis of infections. Binomial logistic regression analysis was performed for estimation of odds ratio (OR) to establish independent risks of extra-pulmonary infections and their recurrences in DM cases compared to non-diabetic controls. P >.05 was considered statistically significant.
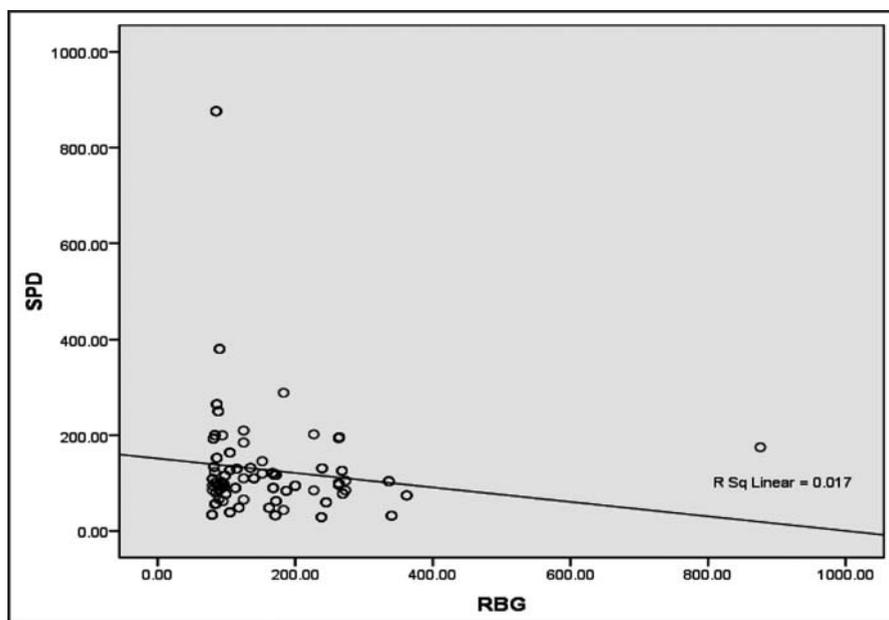
Results
Of the 120 subjects, 60(50%) each were cases and controls. The overall mean age was 45.00±11.6 years, and 82(68.3%) were males. Significant difference was found in mean weight and BMI between the two groups (Table-1).

Serum SP-D levels were significantly lower in cases compared to controls (p=0.036), while RBG was higher in cases (p=0.0002) (Figure-1). There was a negative
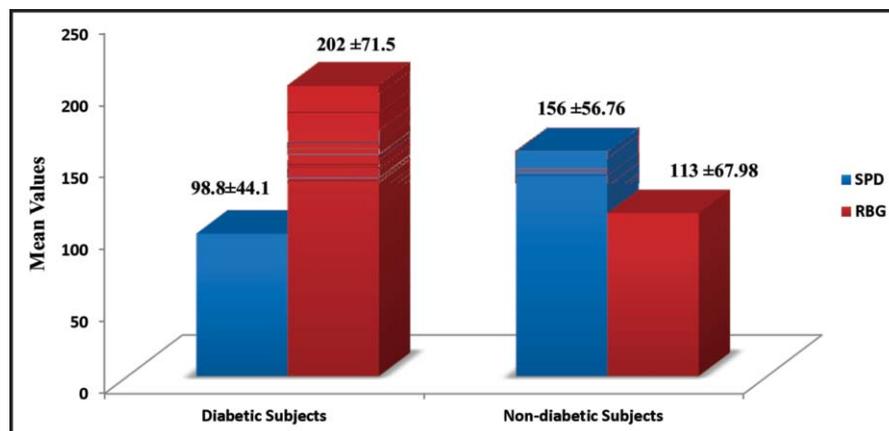
Mean Random Blood Glucose (RBG) is in milligram / deciliter (mg/dl)
P value RBG = 0.0002*
Mean Surfactant protein -D (SPD) is in nano gram /milliliter (ng/ml)
P value SP-D = 0.036*
Statistically Significant difference at P ≤ 0.05

Figure-1: Comparison of biochemical variables among diabetic (n=60) and non-diabetic subjects (n= 60).

The line indicates negative linear relationship resulting from regression between variables.
Beta coefficient (β) = - 0.151, Standard Error (SE) = 0.106,   P value = 0.15
Dependent Variable is Surfactant protein -D (SP-D)
Independent Variable is random blood glucose (RBG)
P value ≤0.05 was considered as Statistically Significant.

Figure-2: Scatter plot showing negative linear relationship between random blood glucose (RBG) and surfactant protein-D (SP-D) of total population (n=120).
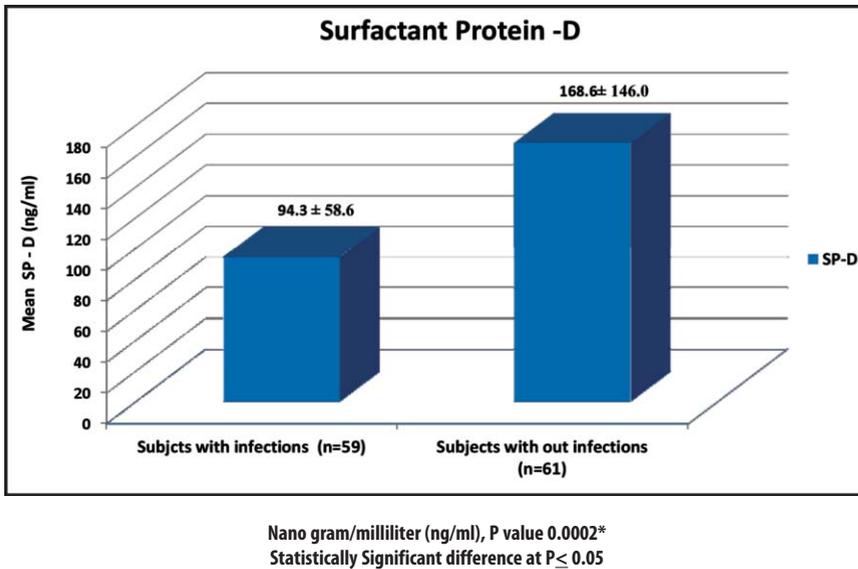
Surfactant Protein -D

Nano gram/milliliter (ng/ml), P value 0.0002*
Statistically Significant difference at P≤ 0.05

Figure-3: Comparison of surfactant protein -D (SP-D) in subjects with and without extra-pulmonary infections (n=120).

Table-1: Basic characteristics of study population.

Parameters	Diabetic subjects (n=60) Mean ± SD	Non-Diabetic Subjects (Control group) (n=60) Mean ± SD	P value
Age (years)	53.80± 6.18	49.2 ±8.83	0.45
Height(meters)	1.63± 0.11	1.65 ± 0.08	0.372
Weight(kg)	73.1± 13.42	60.1± 9.51	0.000*
BMI (kg/m^2)	28.7± 7.19	21.8 ± 2.72	0.000*

BMI = body mass index, Kg = kilogram, Statistically Significant difference at P ≤ 0.05.

Table-2: Binomial logistic regression analysis to evaluate the independent effect of diabetes mellitus on extra pulmonary infections.

Groups	Odd Ratio (OR)	P- Value	95% CI
Diabetic Subjects Vs non Diabetic Subjects	2.10	0.04*	1.01 - 4.35

Statistically Significant difference at P ≤ 0.05; CI: Confidence Interval.

association between SP-D and RBG (Figure-2). Infection rates were significantly higher in cases than controls (p=0.04). Overall, 59(49.1%) subjects had history of extra-pulmonary infections, while 61(50.8%) had no history of recurrent infections.

SP-D levels were lower in subjects with history of recurrent extra-pulmonary infections compared to subjects without history of infections (p=0.0002) (Figure-3). Pearson correlation coefficient was also significant (p=0.0001), indicating significant negative relation between SP-D and infections. The relative risk for extra-

pulmonary infections in diabetic patients was 2 times higher than non-diabetic controls (OR: 2.10, p=0.04).

Discussion

T2DM is an emerging global health issue.[11] Generalised infections more frequently occur in diabetics due to their immuno-compromised state. SP-D is an immuno-modulatory protein that may aid in reducing oxidative stress and regulation of chronic inflammation.[11] SP-D also aids in enhancing bacterial trapping by neutrophil extracellular traps (NETs) during neutrophil-mediated host defense.[19]

It has been postulated that high concentration of glucose and increased activity of elastases in diabetics result in reduction in the biological activity of SP-D and subsequent recurrent pulmonary as well as extra-pulmonary infections. Deficiencies of SP-D have been found to be related with alterations of glucose metabolism. These deficiencies run in parallel with inflammation and infections.[11] Glucose is one of the preferred ligands for carbohydrate recognition domain (CRD) of SP-D and potential inhibitor of the SP-D function in diabetes. High concentration of glucose can interfere with SP-D to interact with broad spectrum of pathogens and abolish neutralisation of different viruses.[12] Moreover, SP-D is subjected to proteolytic degradation by elastases and contributes to reduction in SP-D activity.[1] In contrast to these factors, calcium and protease inhibitor alpha-2 macroglobulin (α_2M) present in serum can abolish SP-D proteolysis and enhance its activity by binding to its domain.[20] High concentration of glucose can compete with the α_2M. There will be an increase in cleavage and inactivation of CRD by elastases because of failure of α_2M and SP-D interaction[2] Evidence shows association of SP-D gene single nucleotide polymorphisms (SNPs) with T2DM.[2]

The current study was planned to compare the SP-D levels among diabetics and age-matched non-diabetics and to correlate SP-D and extra-pulmonary infections in T2DM. It revealed higher rates of recurrent infections, including dermatological, foot infections, genitourinary tract and GIT infections in diabetics compared to the non-diabetics. OR indicated that the diabetic s had 2 times higher relative risk for extra-pulmonary infections than the controls. The findings are in line with earlier stdies done in the United Kingdom[8] and Canada.[9] The

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current study found significantly lower levels of SP-D in diabetics (98.8ng/ml) than non-diabetics (156ng/ml) and this difference was statistically significant. This finding is also in accordance with literature.[12,21] However, contrary to our findings, a recent study documented increased serum SP-D in type 2 diabetics.[22] The discrepancy is most probably because of the fact that they evaluated the relation between lung function and SP-D in diabetics over a long duration of >5 five years. Higher circulating SP-D levels might be because of increased transmigration of SP-D from the alveolar space into the blood due to increased permeability of pulmonary capillaries by chronic inflammation, which may reflect lung injury as a consequence of chronic diabetes.

Although in the current study, significantly lower levels of SP-D in diabetics were observed, the data indicated weak negative association between SP-D and RBG which was not significant. This might have been because of the small sample size. The finding is not in line with the reports from different regions of the world.[11,12,21] The disparity concerning SP-D and blood glucose might be because of small sample size of the current study. The current study found that subjects with history of recurrent infections had decreased SP-D levels in comparison with subjects without infections, indicating a significant negative correlation of SP-D with infections. Deficiency of SP-D leads to reduction in ability of the immune system to eradicate pathogens and may cause frequent infections, and chronic inflammation.[1,3] It is evident that SP-D is on the crossroad of inflammation, glucose intolerance, insulin resistance and diabetes.[12] In future it might be used as an appropriate preventive and therapeutic tool against common infections in immuno-compromised patients.

The current study has limitations. The causal link between diabetes and serum SP-D was not established, and the pathophysiological links involving SP-D, diabetes and infections were not explored. Despite the limitations, the current study remains the first from Pakistan concerning the association of SP-D and RBG.

Limitation
This study was conducted in year 2012-13. Regretfully its publication was delayed.

We speculate that reduced SP-D levels could lead to infections and inflammation in diabetic patients but causal link between diabetes and serum SP-D was not established, additional Cohort studies should be conducted to establish causal link between T2DM and serum SP-D

Conclusion
Patients with diabetes mellitus posed two-time more risk for extra-pulmonary infections and exhibited lower serum SP-D than non-diabetic subjects. SP-D was negatively associated with extra-pulmonary infections. SP-D and RBG were not significantly related.

Disclaimer: None.

Conflict of Interest: None.

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