Original Article

THE STUDY OF RELATIONSHIP BETWEEN THE SERUM LEVELS OF SEX HORMONE-BINDING GLOBULIN (SHBG) AND INCREASED INSULIN RESISTANCE IN THE MALE OFFSPRING OF TYPE 2 DIABETIC PARENTS, RISK OF DEVELOPMENT OF DIABETES IN THEIR OFFSPRING

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ABSTRACT:

OBJECTIVE: To determine prospectively the relationship between insulin resistanceand sex hormone binding globulin(SHBG) in connection with risk ofdeveloping diabetes in male offspring oftype 2 diabetics.

MATERIALS AND METHODS: This cross-sectional, analytical study included 80 subjects, 40 non-diabetic males (20-30years of age) of single type-2 diabeticparents and 40 age matched healthy male controls without a family history ofdiabetes. For each participant, detailedmedical history and clinical examinationwas performed. Fasting serum glucose,insulin, total testosterone, and sexhormone binding globulin weremeasured using standard kits. Bodymass index and insulin resistance indexwere calculated. For data analysis, groups of young adults(with and without family history of type-2 diabetes) were matched using validcriteria for significance of difference.

RESULTS: Mean serum insulinconcentration, insulin resistance, body mass index (BMI) and waist circumference (WC) were significantlyhigher while sex hormone bindingglobulin levels were significantly low instudy subject. Insulin resistancecorrelated inversely with sex hormonebinding globulin.

CONCLUSION: Low levels of sex hormonebinding globulin, hyperinsulinemia andinversecorrelation between sexhormone binding globulin and insulinresistance predict the possibledevelopment of diabetes mellitus inmale offspring of type 2 diabetics.

KEYWORDS: Insulin resistance,Sex Hormone Binding Globulin (SHBG), Type 2 diabetes
In treatment of GDM, glibenclamide is successful in achieving good glycemic control.

INTRODUCTION:

Type 2 Diabetes mellitus accounts for more than 90% of all diabetes cases globally[1]. Epidemiological studies have suggested that the prevalence willcontinue to rise globally without effective prevention and control programs[2].Established risk factors for the development of type 2 DM include obesity, an unfavorable body fat distribution, insulin concentrationand insulinresistance[3].Recent research has shown that, insulin resistance is one of key indicatorsfor development of diabetes in offspring of type 2 diabetics, but exact mechanism is not known[4]. It has also been expressed that low plasmaSHBG levels and SBHG polymorphisms are stronglyassociated and predict the occurrence of type 2diabetes in both genders[5].SHBG plays an important role in regulating the free sex hormones levels and helps in cellular uptake of

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the hormones[6]. It is also reported,that low serum testosterone levels, also predict the developmentof type 2 diabetes. However, any significant association has not been found between testosterone levels and insulin resistance, possibly due to differences in testosterone assessmentand data analysis methodology[7,8,9.]

A strong correlation between SHBG, (which strongly binds andtransports testosterone in the circulation) and insulin resistance, central body fat and dyslipidemia has been observed. Low SHBG levels indicate the increased risk ofdiabetes mellitusas strong independent association between them has been found[7,8].Type 2 diabetics particularly male patients have been studied to find that SHBG strongly associatedwith insulin sensitivity[10].In non-diabetic males,a significant association was too,seen between SHBG and insulin secretory pulse, total and non-oxidative glucosedisposal[11.]

Data isscarceregarding the relationship between insulin resistance and SHBG and potential risk of development of type 2 DM in offspring of type 2 diabetics. The present study was carried out to find out possible role of SHBG in insulin resistance, insulin secretion and whether SHBG and insulin resistance may be a causative factor in development of type 2 DM in children of type 2 diabetics.

RESEARCH DESIGN AND METHODS:

The cross sectional, analytical study was conducted in the Department ofPhysiology, University of Health Sciences, Lahore, in collaboration with diabetes clinic of Allied Hospital, Faisalabad and Independent Medical College andUniversity Trust Hospital, Faisalabad. The study was approved by the Ethicalcommittee and Advance Studies and Research Board of the University of HealthSciences, Lahore.It was a continuous study and was completed in one year. A total of 80 non-diabetic,healthy male subjects between 20-30 years of age were included inthis study. The study population was divided into two groups of forty subjectseach. The control group consisted of offspring of both non-diabetes parentshaving no history of any metabolic disease. The controls were recruited from different colleges, universities and institutions. The study group consisted of offspringhaving one of their parents suffering from type 2 diabetes either father or mother. The subjects were recruited from diabetes clinics of public-sector hospitals. Subjects with endocrine abnormalities such as Cushing's disease, thyrotoxicosis, acromegaly or hypogonadism and those receiving hormone replacement therapy were excluded from the study. All the subjects were asked for written informed consent. Subjectswere initially screened with a questionnaire detailing their complete demographicinformation, medical historyand clinical examination, family history of diabetes,concomitance medication as well as their life style characteristics e.g. physicalactivity, dietary habits, smoking and economic status. A complete physical examination was performed on all subjects thatincluded, recording of pulse, systemic arterial blood pressure (BP), temperature, and systemic examination.

MEASUREMENT OF BLOOD PRESSURE:

Systemic arterial blood pressure was measured by the mercurysphygmomanometer (Certeza CR-2011) in sitting position from the right arm, using an appropriate cuff size. The first and fifth krotokoff sound was recordedas systolic and diastolic B.P respectively.

ANTHROPOMETRIC MEASUREMENTS:

Height (cm) and body weight (kg) were measured by standard procedure tocalculate BMI as weight (kg) divided by height squared meter (m2) as under.

BMI= body weight (kg)/ height (m2)[12]

Waist circumference (WC) was measured using a flexible measuring tape in thehorizontal position, in the middle between 12th rib and iliac crest at the level ofumbilicus during mid-inspiratory phase with the subject breathing normally[13].

BLOOD SAMPLING:

Five ml of venous blood from each subject was drawn between 08:00 and 10:00AM after an overnight fasting of 8-12 hours. Two ml of the

sample was added toan ethylene demine tetra acetic acid (EDTA) tube for glucose estimation. Fastingserum glucose levels were determined within 24 hours of sample collection.Three ml of the blood sample were added in plain serum tubes and werecentrifuged immediately at a speed of 5000 revolution per minute (rpm) for 10 minutes. Following centrifugation, the samples were aliquot and storedimmediately at- 4 C for biochemical analysis.

BIOCHEMICAL ANALYSIS:

SERUM GLUCOSE, INSULIN AND INSULIN RESISTANCE:

Fasting serum glucose level were assayed by a glucose oxidize method usingcommercial reagent (Linear Chroma Spain). The estimations were made withhumastar-180 chemistry analyzer (Human, Wiesbaden, Germany). Serum hormone concentrations were determined using standard immunoassays toestimate the level of serum insulin of individuals in the study. Serum insulin wasmeasured by a commercially available ELISA kit (Aesku, Diagnostics, andWendlsheim, Germany) with an automated EIA analyzer (code; Bio-Redlaboratories Hercules CA, USA. Insulin resistance (HOMA-IR) was calculatedby using fasting serum glucose and insulin levels with the help of the formula.

HOMA-IR=fasting insulin (uU/ml) x fasting glucose (mmol/l) 22.5[14].

Reference range for insulin resistance (HOMA-IR) is considered to be >2.5[15].

Serum total testosterone was measured by chemiluminescent enzyme immunometericassay with the help of VITROS ECIQ immunodiagnostic system which is an automatedrandom access immunodiagnostic assay analyzer based on the principal ofchemiluminescence using commercially available total testosterone calibrator theinstrument.Serum SHBG concentrations were measured by enzyme- linked immunosorbentassay technique (ELISA) using commercially available kit (Diagnostic SystemLaboratories Hercules CA, USA).

STATISTICAL ANALYSIS:

For data analysis, SPSS version 19 (SPSS, inc Chicago, IL USA) was used. Value are expressed as a percentage of each group or as mean + SD unless otherwise stated. Comparisons between groups were made using a student's t-test. Results were considered statistically significant at $p < 0.05$.Shapirwilk test was applied to check the normality of the data. The p- value of allquantitative variable was <0.05, indicating that the data follows normaldistribution and so appropriate statically tests have been applied.

RESULTS:

The study comprised of a total of 80 healthy male subjects, forming two groups; the control and the study group, each heaving 40 subjects.Table 1 summarizes the anthropometric characteristics of control and studygroup (non-diabetic offspring of single diabetes parents).The mean ± SD age of controlgroup was 23.50 ± 3.5 years and of study group was 24.12 ± 2.95 years. Themean ± SD body mass index (BMI) of control group was 20.19 ± 4.06Kg /m2and of study group was 23.30 +3.73 Kg/m^2. The difference in BMI for both group was statically significant ($p<0.001$). The mean ± SD waist circumference was75.66 ± 5.16 cm and 81.45 ± 5.00 cm in control and study group respectively. Thewaist circumference difference was statistically significant between both group; ($p<0.000$).

Parameters	Controls (n=40) Mean ± SD	Study subjects (n=40) Mean ± SD	*p-value*

Table 1.Physical characteristics and anthropometric variables of study population.

**Highly significant. n = number of subjects

Table 2 summarizes the glycemic parameters of control and study group subjects. Nosignificant difference was observed in mean ± SD fasting blood glucoseconcentration of control (4.24±0.71mml/L) and study group (4.37±0.61mmol/L); (p=0.396). The mean ± SD serum insulin concentration was 4.30±2.84

uU/mland 10.25±1.87 uU/ml in control and study groups respectively and wassignificantly higher in subjects of study group than control: (p=0.000). The mean± SD serum insulin resistance (HOMA-IR) was 0.81± 0.53 and 1.99±0.47in control and study groups respectively. The difference between the two groups wasstatistically significant; (p=0.000)

Insulin resistance (HOMA-IR)	0.81 ± 0.53	1.99 ± 0.47	0.000**

Table 2. Glycemic parameters of control and study groups.

**Highly significant. n = number of subjects

Table 3 shows hormonal parameters of control and study group subjects. Themean ± SD serum testosterone concentration was 19.36± 6.15 nmol/1 and12.51± 2.21 nmol/1 in control and study groupsrespectively. The serum testosterone difference between two groups was statistically significant; p=0.000. The difference of serum SHBG concentration in control and study groups was significant as p = 0.000. The mean ± SD values for serum SHBG was 68.40 ± 41.29 nmol/l and 34.74 ± 9.24 nmol/l in control and study groups respectively (table 3).An inverse correlation was found between serum SHBG and insulin resistance (r = - 0.484., p = 0.002).

Table 3.Comparison of serum testosterone and SHBG in study and control groups.

**Highly significant. n = number of subjects

Parameters	Controls (n=40) Mean ± SD	Study subjects (n=40) Mean ± SD	*p-value*
Serum testosterone (nmol/l)	19.36 ± 6.15	12.51 ± 2.12	0.000**
Serum SHBG (nmol/l)	68.40 ± 41.29	34.74 ± 9.24	0.000**

DISCUSSION:

Non-diabetic first degree siblings of type 2 diabetics are more to develop DM and have been studied to identify early metabolic and hormonal abnormalities[16]. In fact, in certain populations, the offspring of type 2 DM parents havehigher risk ofdeveloping DM than those ofnon-diabetics[17]. The main objective of the present study wasto detect,the possible biochemical markerswhich may predictthe developmentof diabetes in children of type 2 diabetics. It has been observed that bothinsulin resistance and low serum SHBG levels, indicate higher risk oftype 2 DM in both sexes[18]. But it is not well established, that low SHBH levels and insulin resistance can also be one of the causative factors in developing type 2 DM in offspring type2 DM parents.

In this study, we observed that non-diabetic male offspring of type-2 diabetics had elevated serum insulin concentration (hyperinsulinemia) and had statistically significanthigh levels of insulin resistance as compared to controls. Similar results were alsoobserved in other studies, indicating that the subjects with progressively stronger familyhistory of diabetes, have shown hyperinsulinemia, supporting insulin resistance hypothesis[7,19]. The current study reported fasting hyperglacemia and normoinsulinemia, possibly due todecreased insulin secretion in the offspring of type-2 diabetics which may bedue to an underlying degree of beta cell dysfunction in this population[20]. Insulinresistance is probably, one of the most likely causative factors in the development of DM inthe offspring of type 2 diabetics[21],but exact mechanism is stillnot fully explored.

It has been observed that insulin resistance probably causes dysregulationsinintra-myocellular fatty acid metabolism in insulin-resistant offspring of type-2 diabetics, because of a genetic defect in mitochondrial oxidativephosphorylation[18]. Studies have foundraised plasma fatty acid levels[17] and intra-myocellular fat content in the insulin-resistant children of type-2 diabetics[22], indicating that insulin resistance may result from abnormalitiesin fatty acidmetabolism in these persons. These observations were in line with our studyresults thatshow that offspring of type-2 diabetics had mean ± SD BMI of 23.30 ±3.73 KG/M2 than controls (20.19 ± Kg/m2) and difference in BMI for both groups is statistically significant; (p= 0.001). Similarly, the waist circumference difference was also statistically significant between the two groups;

(p=0.000).

Insulin has a potent effect on steroid hormone metabolism and it has been shown thatphysiologic increase in serum insulin levels decrease serum adrenal androgens and serum SHBG levels[23]. Little is known about how insulin affects the sex hormone levels, particularly in male offspring of type 2 diabetics. In the present study, significantly lower levels of SHBG (p=0.001) in male offspring of type 2diabetics were found than controls. Moreover, strongly inverse correlation was seen between serum SHBG and insulin resistance (p=0.002) in the study.

The correlation of insulin resistance with steroid hormones and transport proteins is expressedin many cross-sectional studies, proposing that pre-diabetic hyperinsulinemia mightinhibit the production of SHBG[24].It has also been suggested that SHBG may be a makerfor insulin resistance[25]. Serum SHBG concentration is regulated by plasma insulin or insulin resistance so elevated insulin levels orinsulin resistance are correlated with lower SHBG levels[26]. Theseobservations are quite similar to current study results, which also found highly significant inverse correlation between insulin resistance and SHBG; high serum insulin (hyperinsulinemia)and low SHBG. Birkeland observed a strong direct correlation between insulinsensitivity and SHBG in men with type 2 DM[27]. Haffner et al reported rather moreconsistent evidence of occurrence of DMs with higherinsulin and low SHBG levels and relatively inconsistent risk of diabetes over varying levels of total testosterone[28].Our study results exhibited hyperinsulinemia, insulin resistance andsignificantly lower levels of SHBS and total testosterone in study subjects, which is themainstay of this study.

CONCLUSION:

Our study concluded that the offspring of type 2 diabetic parentsare more prone to develop diabetes because of presence of insulin resistance and hypeinsulinaemia. There is a negativecorrelation between SHBG and insulin resistance in study subjects making the offspring more susceptible to develop diabetes.

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