Original Article

ROLE OF SEX HORMONE BINDING GLOBULIN AS AN INDICATOR OF INSULIN RESISTANCE

Nayab Zehra, Muhammad Usman Munir, Muhammad Qaiser Alam Khan, Zujaja Hina Haroon, Mohammad Younas, Muhammad Anwar

Armed Forces Institute of Pathology (National University of Medical Sciences), Rawalpindi Pakistan

ABSTRACT

Objective: To determine the role of Sex Hormone Binding Globulin (SHBG) in cases of hyperinsulinemia and insulin resistance among our population.

Material and Methods: It was a cross-sectional analytical study conducted at Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, from Jun 2022 – Dec 2022**.** Study is comprised of 90 samples consisting of both males and females. Study individuals were categorized as Pre-Diabetic and Diabetic according to their fasting plasma glucose levels. Fasting plasma insulin levels were done on Advia Centaur XPT Chemiluminescence Immunoassay analyser. Serum SHBG was done on Immulite 2000 by chemiluminescent immunometric assay. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated from fasting glucose and insulin levels. Cut off value of 2.2 was used for labelling insulin resistance. Data was analyzed on SPSS version 23. Mean and standard deviation (SD) was calculated for glucose, insulin and SHBG. T-test was used to compare means of pre diabetic and diabetic groups. Pearson's correlation was used to establish correlation between SHBG and HOMA-IR.

Results: Our study included 57 males and 33 females with mean age of 47±11 years and 47±12 years, respectively. SHBG levels were significantly higher in pre-diabetics as compared to diabetics. The Pearson's correlation between SHBG and Fasting serum insulin is weak negative (r = 0.3, p = -0.23), same is between SHBG and HOMA-IR (r = 0.25, p = -0.86), showing no significant association between SHBG and HOMA-IR among our population. However, two tailed t-test showed marked difference between means of serum SHBG, fasting serum insulin and HOMA-IR among diabetic and pre-diabetic groups with 95% confidence Interval.

Conclusion: The negative association of SHBG with insulin resistance is not marked in our population. Various epidemiological, external and metabolic factors could affect and should be excluded before establishing this negative correlation between SHBG and Insulin resistance for predicting the development of diabetes mellitus (DM)

Key Words: SHBG, Hyperinsulinemia, HOMA-IR, Correlation

This article can be cited as: Zehra N, Munir MU, Khan MQA, Haroon ZH, Mohammad Younas M, Anwar M. Role of sex hormone binding globulin as an indicator of insulin resistance. Pak J Pathol. 2023; 34(1): 6-10.

DOI: 10.55629/pakjpathol.v34i1.747.

INTRODUCTION

Sex hormone – binding globulin (SHBG) is a protein having molecular weight of 90 –100 kDa produced mainly by the liver. The short arm of chromosome 17 resides its gene. Main function of SHBG is to transport testosterone (T), estradiol (E2) and some other steroids in the blood reducing their metabolic rate and enhancing approach to the target tissues. SHBG not only acts as a binding and a transportation hormone but also exerts some cellular effects by regulating the target response of sex hormones [1]. Moreover, it has been shown that insulin might exert inhibitory effects on SHBG production [2]. In accordance with these findings, obesity and insulin resistance have been found related with the decreased level of SHBG [3].

Correspondence: Dr Nayab Zahra, Registrar, Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (National University of Medical Science), Rawalpindi Pakistan.

Email: **nayabhussain9@gmail.com**

Received: ***06 Dec 2022****; Revised:* ***22 Feb 2023****; Accepted:* ***07 Mar 2023***

A study conducted by Veltman et.al (2010) also showed an association of low levels of SHBG in females with gestational diabetes mellitus (GDM) and polycystic ovaries depicting an association of SHBG with body metabolism and its hormonal profile. The negative correlation between SHBG and hyperinsulinemia points towards development of diabetes mellitus may be due to various impending factors responsible for this association such as dietary factors which may influence SHBG gene transcription [4].

A study conducted by Wallace IR *et al* (2013) has provided new vision of the function and regulation of human SHBG. Based on these findings, it was hypothesized that SHBG could be a valuable parameter of both metabolic and endocrine disorders [5]. Serum level of SHBG is a matter of hormonal, metabolic, genetic and nutritional regulation which may also lead to insulin resistance resulting in diabetes. Fatty enlargement of liver and insulin resistance are important factors for establishing gene expression of SHBG and these determinants explain

why low levels of SHBG are linked with type 2 diabetes [6]. Low levels of SHBG are also associated with type 2 diabetes, metabolic syndrome as well as cardiovascular diseases which may go along with these conditions. On the other hand, SHBG levels increase after reducing weight, enhancing insulin sensitivity [7,8].

The relationship between SHBG and serum fasting insulin or C-peptide levels has been established demonstrating improvement in insulin resistance (IR) with reduction in weight or following treatment with insulin-sensitizing medications [19]. Presently, SHBG has appeared as a new biomarker for IR. However, a limited data is available in Asian population. So, the exact mechanism for this correlation still needs to be discovered. Keeping in view the ethnic differences from other population we decided to determine the relation between SHBG and insulin levels in our population. Moreover, our study will help in elaborating role of SHBG in cases of hyperinsulinemia and developing insulin resistance in our population having demographic, racial and metabolic differences.

MATERIAL AND METHODS

It was a cross-sectional study, conducted at Armed Forces Institute of Pathology, Rawalpindi / National University of Medical Sciences after approval from Institutional Review Board. Taking 10% prevalence of prediabetes (Insulin resistance) in our population having confidence level of 95% with margin of error 6%, we calculated sample size to be 90 to include in our study [10]. Glucose concentration in the range of 5.6 – 6.9 mmol/L was taken as pre-diabetes while diabetes was labelled at glucose concentration ≥ 7 mmol/L, according to American Diabetes Association (ADA) Guidelines.[11] Study individuals were grouped in Pre-diabetics and Diabetics according to their fasting plasma glucose levels. All patients on exogenous insulin were excluded.

Venous sample (3 ml) for plasma glucose fasting was taken in sodium fluoride tube which was analysed on Advia 1800 Clinical Chemistry Analyzer. Fasting serum sample (2ml) of insulin was taken in gel-tube simultaneously analysed on Advia Centaur XPT Immunoassay auto- analyser by chemiluminescence method having Analytical Measuring Range (AMR) 0.5-300 mU/L. SHBG serum sample (1 ml) taken in gel tube was run on Immulite 2000 by chemiluminescent immunometric assay having analytical sensitivity of 0.02 nmol/L. Samples centrifugation was done at 3500 rpm for three minutes and were analysed within 2 hours of collection.

Three levels of controls including low, normal and high were run with each batch of plasma glucose, serum insulin and serum SHBG as a part of internal quality control. Z score of insulin for External Quality Control (EQAS) was < 2.0 during the study period. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated from fasting glucose and insulin levels. Cut of value of 2.2 was be used for labelling insulin resistance [12].

Statistical Package for Social Sciences (SPSS) version 23 was used for all statistical calculations. Mean and standard deviation (SD) was calculated for glucose, insulin and SHBG. One sample t-test was applied to compare means of pre diabetic and diabetic groups. Pearson's correlation was used to establish correlation between SHBG and HOMA-IR. All statistical tests were two-sided. $p < 0.05$ is considered statistically significant.

RESULTS

Our study includes 57 males and 33 females with mean age (Mean ± SD) of 47 ± 11 years and (Mean ± SD) 47 ± 12 years, respectively. We divide the study population according to their fasting serum insulin and HOMA-IR values in 2 groups. Serum insulin levels were found to be low in pre-diabetic population as compared to diabetic group indicating more insulin resistance among diabetics. However, SHBG levels were significantly higher in pre-diabetics as compared to diabetics. The Pearson's correlation between SHBG and serum insulin fasting came out to be weak negative (r = 0.3, p = -0.23), same as between SHBG and HOMA-IR (r = 0.25, p = -0.86) as shown in Table-I. Age distribution between pre-diabetics and diabetics is shown in Table-II having more participants in age bracket of 30-45 years. However, more male participants (63%) were present in the study than females (37%).

Two tailed t-test showing marked difference between means of serum SHBG, Serum insulin fasting and HOMA-IR among diabetic and pre-diabetic groups.

Table-I: SHBG and HOMA-IR among Pre-diabetics (n=58) and Diabetics (n=32), (Total n=90)

Variables	Pre-Diabetics Mean ± SD (n=58)	Diabetics Mean ± SD (n=32)	*p*- value (< 0.05)
Fasting Plasma Glucose (mmol/L)	6.09 ± 0.33	10.07 ± 3.5	< 0.001
Fasting Serum Insulin (U/L)	9.61 ± 9.18 (1.90-34.32)	14.39 ± 12.22	0.038
HOMA-IR	2.7512 ± 2.63	6.5606 ± 5.87	<0.001
SHBG (nmol/L)	35.30±29.14 (5.50-180.0)	23.65±11.02	0.032

SHBG; Sex Hormone Binding Globulin, SD. Standard Deviation, HOMA-IR; Homeostatic Model for Assessment of Insulin Resistance

Table-II: Age distribution among Pre-diabetics and Diabetics (Total n=90)

Age in Years	Pre-diabetics (5.6-6.9mmol/L)	Diabetics >7.0 mmol/L	Total (n)
30-45	35 (38%)	08 (8.8%)	43 (47.7%)
46-60	11 (12.2%)	21 (23.3%)	32 (35.5%)
61-75	12 (13.3%)	03 (3.3%)	15 (16.6%)

DISCUSSION

SHBG is an important globulin (protein) not only for transport of sex hormones but also for regulation of multiple metabolic pathways among several endocrine functions. It is mainly produced in the liver under various hormonal and dietary factors. It has been suggested that low levels of SHBG are usually found in cases of insulin resistance relating that its secretion is inhibited by insulin, hence SHBG has been considered as an effective predictive marker for development of diabetes [6]. However, little data is available in Asian population regarding this emerging marker of insulin resistance. The hormonal profile may show variations in different population because of varying environmental factors and various metabolic needs of the body. These may be due to variations in ethnicity, gender differences and various socioeconomic variables.

Although, the negative correlation between SHBG and HOMA-IR was found but it didn't come out to be significant. Therefore, we found that low SHBG could point towards developing insulin resistance or metabolic syndrome in both genders but it could be influenced by many external as well as internal factors. Our study showed that in diabetics, the levels of SHBG are lower than in subjects with pre-diabetes. It could be due to low grade ongoing inflammation in diabetes which effects SHBG levels. SHBG has anti-inflammatory effects but in diabetes due to slow inflammatory cascade this effect is masked and SHBG levels are also affected [13].

In one study, Manal Abdalla et.al (2017) also found an association of SHBG with development of diabetes among pregnant ladies. It was concluded that Low serum SHBG is linked with the complications and prognosis of gestational diabetes which plays an important part in pathogenesis leading to Insulin Resistance [14]. Combined with these outcomes, it was suggested that insulin might exert inhibitory effect in the production of SHBG after excluding exogenous insulin and other environmental factors.

Although SHBG has been introduced as an important endocrinological and genetic marker which could possibly contribute to the development of diabetes mellitus (DM), genetic studies also disclose that transmission of a definite polymorphisms in the *SHBG* gene also influences its expression [15]. In another study conducted in Sweden, concentrations of SHBG were inversely associated with log transformed HOMA-IR in all groups [16].

It is obvious from literature review that SHBG might has a role in glucose homeostasis in humans. However, a study on Japanese-American population concluded that SHBG was not a substantial risk factor in either gender, suggesting that there are some ethnic differences which could influence SHBG expression. The mean SHBG concentration was higher in the non-diabetic group (42.2 nmol/L) than the diabetic group (30.5 nmol/L). An inverse but weak association between insulin resistance and SHBG was observed (r = 0.353, $p < 0.015$) [17]. This study also supports our hypothesis that although an inverse correlation exists between SHBG and insulin resistance, this relationship may be influenced by racial and metabolic differences among populations.

A study conducted in China, having 396 participants, all were healthy girls at baseline age 11 years concluded that in early puberty, decline in SHBG predicts development of insulin resistance, independent of adiposity [18]. We also divide our study group in three age groups showing highest number of participants in 30-45 years of age with more in pre-diabetic range having high SHBG concentration.

The results of our study are not exactly consistent with previous studies, indicating that in our population the association between SHBG and insulin was negative but not much significant. As HOMA-IR is also representative of insulin resistance it requires both fasting insulin and fasting glucose levels. On the other hand, exogenous insulin could also be an interferent in measuring insulin resistance so SHBG could also be added in screening patients for insulin resistance after excluding ethnic differences and other co-morbid inflammatory conditions. Hence, the possible direct effects of SHBG in these processes need to be investigated in more detail predicting effects of hormonal imbalance in diabetic and pre-diabetic people. In addition, these results will contribute to the understanding of role of SHBG in diabetes mellitus.

CONCLUSION

The negative association of SHBG with insulin resistance is not marked in our population. Various epidemiological, external and metabolic factors could affect and should be excluded before establishing this negative correlation between SHBG and Insulin resistance for predicting the development of DM.

AUTHOR CONTRIBUTION

Nayab Zehra: Writeup, literature review, sample collection and analysis
Muhammad Usman Munir: Supervision, Data interpretation, statistical analysis
Muhammad Qaiser Alam Khan: Critical review and approval of study
Zujaja Hina Haroon: Revisions
Mohammad Younas: Literature review
Muhammad Anwar: Proofreading

REFERENCES

1. Heald AH, Anderson SG, Ivison F, Riste L, Laing I, Cruickshank JK & Gibson JM. Low sex hormone binding globulin is a potential marker for the metabolic syndrome in different ethnic groups. Exp Clin Endocrinol Diabetes. 2005; 113: 522–8. DOI:10.1055/ s-2005-865807
2. Daka B, Rosen T, Jansson PA, Råstam L, Larsson CA, Lindblad U. Inverse association between serum insulin and sex hormone-binding globulin in a population survey in Sweden. Endocrine Connections. 2013; 2(1): 18–22.
3. Selva DM, Hogeveen KN, Innis SM, Hammond GL. Monosaccharide induced lipogenesis regulates the human hepatic sex hormone binding globulin gene. J Clin Invest. 2007; 117: 3979–87.
4. Veltman-Verhulst SM, van Haeften TW, Eijkemans MJC, de Valk HW, Fauser BCJM, Goverde AJ. Sex hormone-binding globulin concentrations before conception as a predictor for gestational diabetes in women with polycystic ovary syndrome. Hum Reprod. 2010; 25(12): 3123–8. DOI: http://dx.doi.org/10.1093/humrep/deq27.
5. Wallace IR, McKinley MC, Bell PM, Hunter SJ. Sex hormone binding globulin and insulin resistance. Clin Endocrinol (Oxf). 2013; 78(3): 321–9. DOI: http://dx.doi.org/10.1111/cen.12086
6. Sex Hormone-Binding Globulin Gene Expression and Insulin Resistance Stephen J. Winters, Jyothi Gogineni, Marjan Karegar, Charles Scoggins, Chris A. Wunderlich, Richard Baumgartner, and Dushan T. Ghooray Division of Endocrinology, Metabolism and Diabetes (S.J.W., J.G., M.K., D.T.G.), Division of Surgical Oncology (C.S.), Clinical Pathology Associates, Norton Healthcare (C.A.W.), and Department of Epidemiology and Population Health (R.B.), University of Louisville, Louisville, Kentucky 4020
7. Hammoud A, Gibson M, Hunt SC, Adams TD, Carrell DT, Kolotkin RL, *et al*. Effect of Roux-en-Y gastric bypass surgery on the sex steroids and quality of life in obese men. J Clin Endocrinol Metab. 2009; 94(4): 1329–32. DOI: http://dx.doi.org/10.1210/jc.2008-1598
8. Brand JS, Van Der Tweel I, Grobbee DE. Emmelot-Vonk MH, van der Schouw YT. Testosterone, sex hormone-binding globulin and the metabolic syndrome: A systematic review and meta-analysis of observational studies. Intl J Epidemiol. 2011; 40: 189–207.
9. Le TN, Nestler JE, Strauss JF 3rd, Wickham EP. Sex hormone-binding globulin and type 2 diabetes mellitus. Trends Endocrinol Metab. 2012; 23(1): 32-40. DOI: 10.1016/j.tem.2011.09.005.
10. Aamir AH, Ul-Haq Z, Fazid S, Shah BH, Raza A, Jawa A, Mahar SA, *et al*. Type 2 diabetes prevalence in Pakistan: what is driving this? Clues from subgroup analysis of normal weight individuals in diabetes prevalence survey of Pakistan. Cardiovasc Endocrinol Metab. 2020; 9(4): 159-64. DOI: 10.1097/XCE.0000000000000212.
11. https://diabetes.org/about-us/statistics/cost-diabetes
12. Salgado AL, Carvalho LD, Oliveira AC, Santos VN, Vieira JG, Parise ER. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. Arquivos de Gastroenterologia. 2010; 47(2): 165-9. DOI: 10.1590/ s0004-28032010000200009.
13. Liu N, Feng Y, Luo X, Ma X, Ma F. Association between dietary inflammatory index and sex hormone binding globulin and sex hormone in U.S adult females. Front Pub Health. 2022; 10: 802945. DOI: 10.3389/fpubh.2022.802945.
14. Tawfeek MA, Alfadhli EM, Alayoubi AM, El-Beshbishy HA, Habib FA. Sex hormone binding globulin as a valuable biochemical marker in predicting gestational diabetes mellitus. BMC Womens Health. 2017 Mar 9;17(1):18. DOI: 10.1186/s12905-017-0373-3
15. Xing C, Zhang J, Zhao H, He B. Effect of Sex Hormone-Binding Globulin on Polycystic Ovary Syndrome: Mechanisms, Manifestations, Genetics, and Treatment. Int J Womens Health. 2022 Feb 2;14:91-105. DOI: 10.2147/IJWH.S344542
16. Ottarsdottir K, Hellgren M, Bock D, Nilsson AG, Daka B. Longitudinal associations between sex hormone-binding globulin and insulin resistance. Endocr Connect. 2020; 9(5): 418-25. DOI: 10.1530/EC-20-0141

17. Abbiyesuku FM, Agbakwuru AN, Olawale OO. The association between sex hormone-binding globulin and type 2 diabetes in Nigerian men. Afr J Lab Med. 2013. 24; 2(1): 44. DOI: 10.4102/ajlm.v2i1.44

18. Le S, Xu L, Schumann M, Wu N, Törmäkangas T, Alén M, Cheng S, *et al*. Does sex hormone-binding globulin cause insulin resistance during pubertal growth?, Endocrine Connections. 2019; 8(5): 510-17. DOI: 10.1530/EC-19-0044