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[Aleksei Igorevich Ryzhkov](#)^{*}, Svetlana Yuryevna Sokolova, Alexandra Evgenievna Vasilyeva, Igor Sergeevich Shormanov

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Article

Relationship between Serum Concentration of Sex Hormone Binding Globulin, Age, and Testosterone Levels in Men

Aleksei Ryzhkov *, Svetlana Sokolova, Alexandra Vasilyeva and Igor Shormanov

Yaroslavl State Medical University, Mother and Child Clinic Yaroslavl, Yaroslavl; ntvbyfntnrf@mail.ru (S.S.); sashavasileva000@gmail.com (A.V.); i-s-shormanov@yandex.ru (I.S.)

* Correspondence: 1129682@gmail.com; Tel.: +79201129682

Abstract: Background/Objectives: An age-associated increase in serum levels of sex hormone binding globulin (SHBG) is considered to be one of the factors leading to the development of male hypogonadism. Aim of the study: to study the relationship between the concentration of sex hormone-binding globulin in the blood serum and the age of the man, the level of total and free testosterone. A single-center retrospective cross-sectional study was conducted. The results of laboratory tests of 162 male patients aged from 18 to 56 years (median - 34 years) were analyzed. **Methods:** The level of total testosterone and sex hormone binding globulin (SHBG) was determined by chemiluminescent immunoassay. The level of free testosterone was determined by the calculation method according to Vermeullen. Statistical analysis was performed using SPSS Statistics v23. **Results:** Correlation analysis did not reveal a significant relationship between age and SHBG levels, as well as between age and total testosterone levels. Serum SHBG concentration showed a significant ($p < 0.001$) strong positive ($r = 0.61$) correlation with the total level and a significant ($p = 0.042$) but very weak positive ($r = 0.162$) correlation with the level of free testosterone. Comparison of free testosterone levels in groups of patients with SHBG levels below 16 nmol/l, from 16 to 69 nmol/l and above 69 nmol/l did not reveal significant differences ($p = 0.163$). All patients with free testosterone levels less than 225 pmol/l have total testosterone levels below 12 nmol/l. Among men with total testosterone less than 12 nmol/L, only 27% had free testosterone levels below 225 pmol/l. **Conclusions:** Serum SHBG levels in men 18-56 years old are not significantly associated with age. An increase in serum SHBG concentration in men is accompanied by an increase in the level of total testosterone, but does not have a significant effect on the concentration of free testosterone.

Keywords: sex hormone binding globulin; SHBG; male hypogonadism; free testosterone

1. Introduction

An age-associated increase in serum levels of sex hormone-binding globulin (SHBG) has been considered by several authors as one of the factors leading to the development of male hypogonadism [1, 2]. It is hypothesized that an increase in SHBG levels in the blood leads to a decrease in free testosterone concentration, while the total testosterone level may remain within the reference range [3]. We were unable to find convincing studies confirming this point of view. Furthermore, this opinion contradicts the most compelling current concept explaining the physiology of steroid hormones - the "free hormone hypothesis," according to which only the free fraction of steroid hormones can penetrate cells and exert biological effects [4]. This hypothesis suggests that only free testosterone is capable of exerting an antigonadotropic effect, i.e., influencing the production of luteinizing hormone. Accordingly, the functioning of the negative feedback mechanism is directed at maintaining a stable concentration of free, rather than total testosterone. Therefore, under conditions of a normally functioning hypothalamic-pituitary-gonadal axis, the concentration of free testosterone should remain normal at any SHBG concentration. To clarify the role of SHBG in the development of male hypogonadism, we conducted our own study aimed at investigating the relationship between

serum sex hormone-binding globulin concentration, age of the male, and levels of total and free testosterone in the blood.

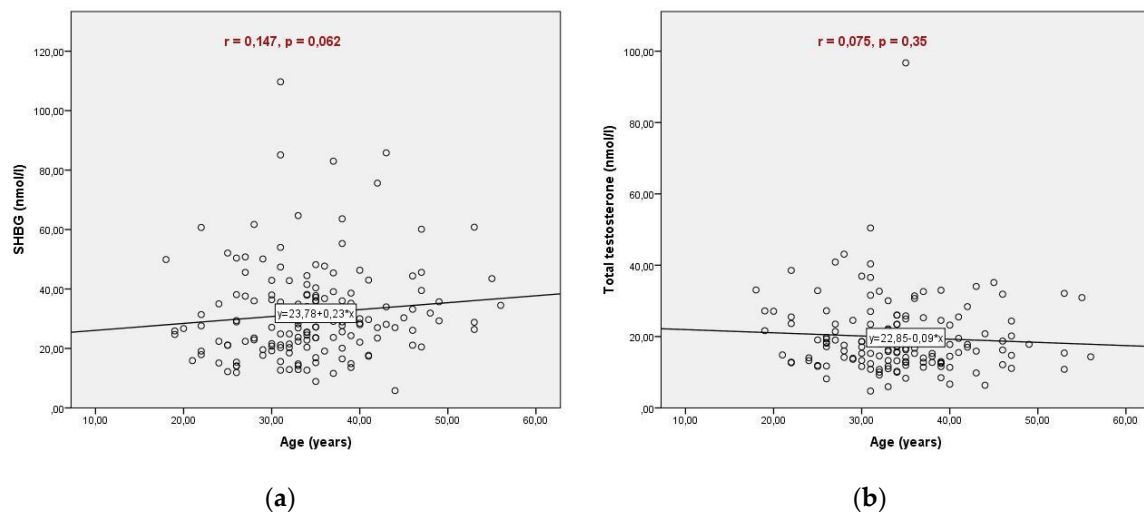
2. Materials and Methods

A single-center retrospective cross-sectional study was conducted. The results of laboratory tests of 162 male patients aged 18 to 56 years (median age - 34 years) who were examined at the "Mother and Child Yaroslavl" clinic from April 2022 to November 2023 were analyzed. The levels of total testosterone (normal range 12-30 nmol/L) and sex hormone-binding globulin (SHBG) (normal range 16-69 nmol/L) were determined by chemiluminescent immunoassay (CLIA). The level of free testosterone (normal range 225-900 pmol/L) was calculated using the Vermeullen method [5]. Statistical analysis was performed using SPSS Statistics v23. Normality of distribution was assessed using the Kolmogorov-Smirnov test. Differences between patient subgroups were evaluated using the Mann-Whitney test (for 2 groups) and Kruskal-Wallis test (for 3 or more groups). Correlation analysis was conducted using the Spearman correlation coefficient. To build a linear regression, the distribution of the dependent variable was normalized by removing outliers. Differences were considered significant at $p < 0.05$.

3. Results

The level of sex hormone-binding globulin in the examined men varied in the range from 5.8 to 109.7 (median 28.9, interquartile range from 21.15 to 37.85) nmol/l. The total testosterone level ranged from 4.7 to 50.4 (median 17.83, interquartile range from 13.26 to 23.44) nmol/l. The calculated free testosterone ranged from 0.342 to 0.503 nmol/l (median 0.396, interquartile range from 21.15 to 37.85) nmol/l.

Correlation analysis did not reveal significant relationships between age and SHBG levels, as well as between age and total testosterone levels, but a significant ($p=0.01$) negative correlation between age and free testosterone levels was found (Figure 1). The correlation coefficient was 0.205, corresponding to a very weak relationship on the Chaddock scale.



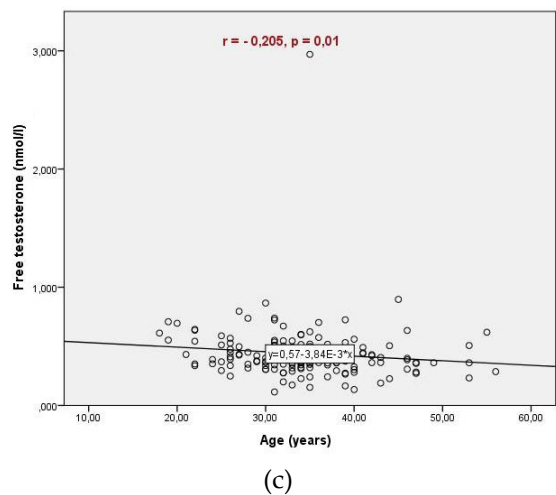


Figure 1. The results of the correlation analysis between the age and the serum level of SHBG (a), total testosterone (b) and free testosterone (c).

To clarify the relationship between age of men and SHBG levels, we divided patients into 7 subgroups based on age: 18-24 years (n=13), 25-29 years (n=23), 30-34 years (n=51), 35-39 years (n=40), 40-44 years (n=17), 45-49 years (n=12), and 50-56 years (n=5) and compared the values of serum SHBG concentration in these groups (Figure 2).

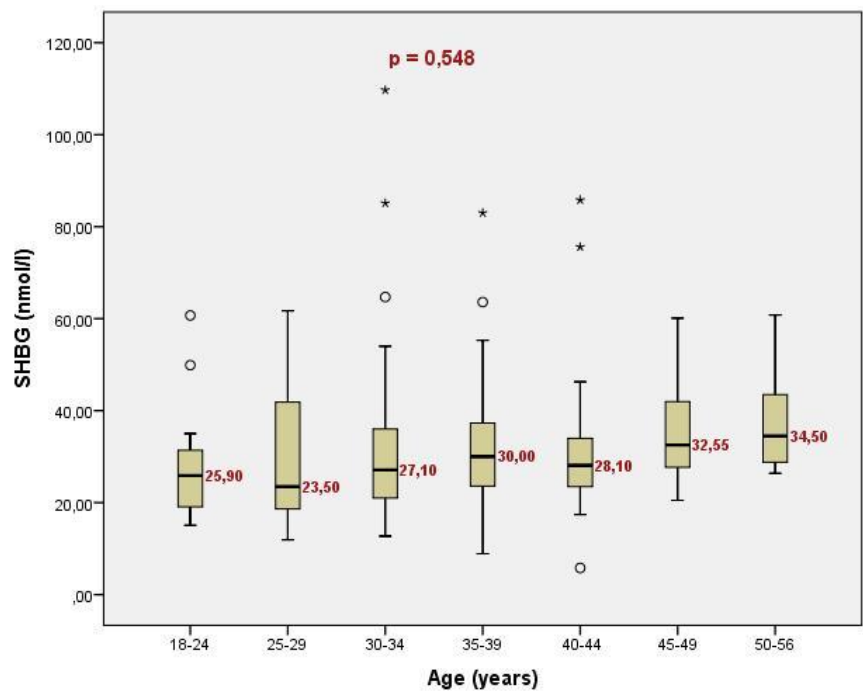


Figure 2. Values of blood SHBG levels in different age groups.

No significant differences in SHBG levels were found between these groups ($p = 0.548$), which, combined with the results of correlation analysis, indicates no association between SHBG levels and age among men aged 18-56 years.

The evaluation of the relationship between serum SHBG concentration and total and free testosterone levels revealed a significant positive correlation between SHBG concentration and both total ($p < 0.001$) and free testosterone ($p = 0.042$) (Figure 3).

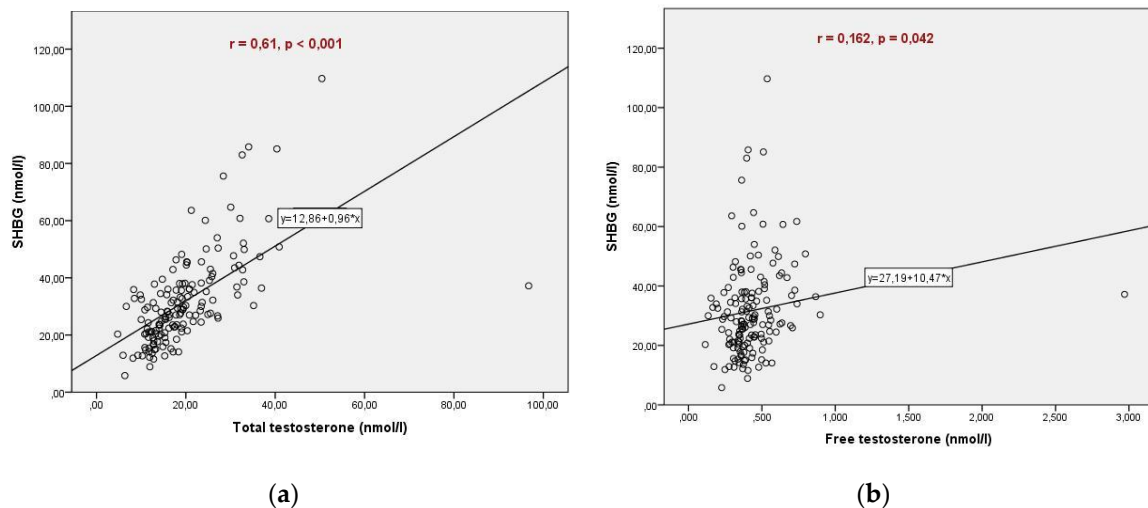


Figure 3. Correlation between serum SHBG concentration and total (a) and free (b) testosterone levels.

The correlation with total testosterone was strong (correlation coefficient of 0.61). Linear regression analysis resulted in a statistically significant model ($p < 0.001$), indicating that a 1 nmol/L increase in SHBG concentration leads to a 0.275 nmol/L increase in total testosterone concentration. The R^2 coefficient for the model was 0.358, suggesting the model is not entirely acceptable.

For free testosterone, the correlation was very weak, and the significance of the differences was close to the threshold, indicating no association between SHBG levels and free testosterone concentration. These findings suggest that an increase in blood SHBG concentration leads to an increase in total testosterone levels but does not decrease free testosterone concentration.

To assess the impact of abnormal SHBG concentrations on total and free testosterone levels, patients were divided into 3 groups: those within the reference range (16-69 nmol/L) ($n=136$), below 16 nmol/L ($n=20$), and above 69 nmol/L ($n=6$). No significant differences in free testosterone concentration were found between patients with normal and abnormal SHBG values ($p=0.163$) (Figure 4).

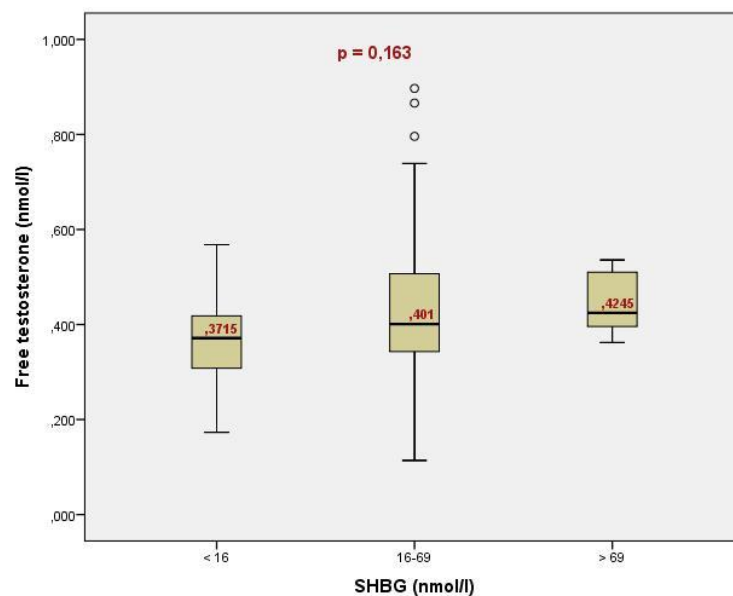


Figure 4. Free testosterone levels in patients with normal and abnormal SHBG values.

However, total testosterone values in these groups showed significant differences ($p < 0.0001$) (Figure 5). Patients with low SHBG had lower total testosterone levels compared to those with normal SHBG, while those with abnormally high SHBG had higher total testosterone levels than men with

normal SHBG values. These results confirm that SHBG concentration influences total but not free serum testosterone levels.

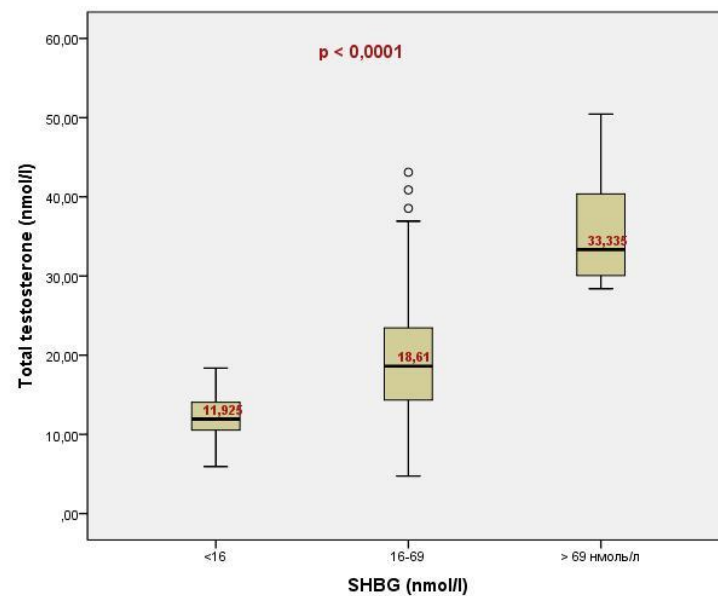


Figure 5. Total testosterone levels in patients with normal and abnormal SHBG values.

Total testosterone levels differed significantly between patients with free testosterone levels above ($n=147$) and below ($n=7$) 225 pmol/L ($p<0.0001$). In all patients with decreased free testosterone, total testosterone levels were below 12 nmol/L (Figure 6).

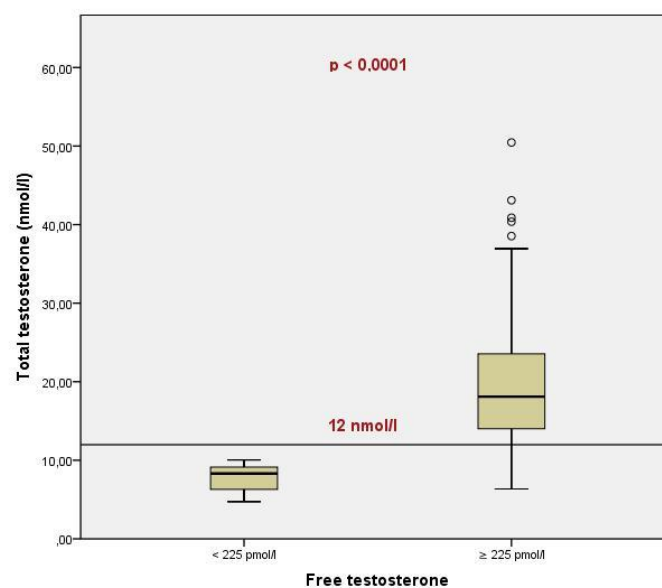


Figure 6. Total testosterone levels in patients with free testosterone levels above and below 225 pmol/L.

Moreover, among men with total testosterone less than 12 nmol/L ($n = 26$), 73% ($n=17$) had free testosterone concentrations above the lower limit of the reference interval, while 27% ($n=9$) had concentrations below. These findings suggest that assessing SHBG levels and calculating free testosterone is advisable only for patients with decreased total testosterone levels.

4. Discussion

In the present study, we did not find an association between serum SHBG levels and age in men aged 18-56 years, although several large studies have reported an increase in SHBG concentration

with age. Muller M. et al. observed a 1.1% increase in blood SHBG concentration per year in men aged 40 to 80 years [6]. Liu P.Y. estimated an annual increase in blood SHBG of 2.3-2.5% on average in men aged 18-90 years, with a more significant annual increase in SHBG observed in older men compared to younger men [7]. In our study, more than half of the men were younger than 35 years, two-thirds were younger than 40 years, and there were no elderly men. The age structure of the sample likely explains the lack of association between SHBG and age in our study.

We found a strong positive correlation between SHBG and total testosterone levels in our study. The influence of SHBG concentration on free testosterone levels in our study was very weak, which we interpreted as a lack of association between SHBG levels and free testosterone concentration. This result is consistent with studies that have examined populations of men with high SHBG concentrations due to SHBG gene polymorphism, showing that an increase in serum SHBG concentration is associated with an increase in total testosterone concentration, but free testosterone concentration does not change significantly [8, 9].

Interestingly, none of the patients with a total testosterone level above 12 nmol/l had a reduced concentration of free testosterone, while 27% of men with a total testosterone level below 12 nmol/l had reduced concentrations of free testosterone. Similar data were obtained in the European Male Ageing Study (EMAS), where an examination of 3068 men aged 40 to 79 years showed that low concentrations of free testosterone were observed in only 27.3% of men with a total testosterone level below 12 nmol/l [10]. The same study also showed that it is low concentrations of free, rather than total, testosterone that are associated with symptoms of hypogonadism [10]. In this regard, it seems advisable to recommend measuring SHBG levels and calculating free testosterone levels in patients with reduced total testosterone levels. This will help avoid overdiagnosis of hypogonadism, as not all patients with reduced total testosterone will have low concentrations of free testosterone and symptoms of hypogonadism. On the other hand, there is no need to test free testosterone in men with normal total testosterone values.

5. Conclusions

The serum level of SHBG in men aged 18-56 years is not significantly associated with age. An increase in serum SHBG concentration in men is accompanied by a rise in total testosterone levels, but it does not have a substantial impact on free testosterone concentration. It is advisable to measure SHBG levels and calculate free testosterone in patients with a total testosterone level below 12 nmol/L, as when total testosterone levels are above 12 nmol/L, the concentration of free testosterone always corresponds to normal values, whereas when total testosterone levels are below 12 nmol/L, reduced free testosterone concentration is observed in only 27% of patients.

Author Contributions: Conceptualization, Aleksei Ryzhkov and Igor Shormanov; Data curation, Alexandra Vasilyeva; Formal analysis, Alexandra Vasilyeva; Investigation, Alexandra Vasilyeva; Methodology, Aleksei Ryzhkov; Project administration, Aleksei Ryzhkov; Resources, Svetlana Sokolova; Software, Aleksei Ryzhkov; Supervision, Svetlana Sokolova; Validation, Aleksei Ryzhkov and Svetlana Sokolova; Visualization, Aleksei Ryzhkov; Writing – original draft, Aleksei Ryzhkov; Writing – review & editing, Igor Shormanov.

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Informed Consent Statement: Patient consent was waived due to the study is associated only with laboratory data without the ability to identify patients.

Data Availability Statement: Dataset available on request from the authors

Conflicts of Interest: The authors declare no conflicts of interest.

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