Original Article

Relationship of Testosterone with White Blood Cells in Adult Males

Farooq Munfaet Ali Khan[1], Muhammad Omar Shamim[2], Rashad Mahmood[4] and Khuram Shahzad Khan[3]

ABSTRACT

Objective: To establish a probable relationship of serum testosterone with total leucocyte count and differential leucocyte count in Pakistani male population.

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the Institute of Basic Medical Sciences, Dow University of Health Sciences, Karachi, lasting from September 2010 to September 2011.

Materials and Methods: It was conducted on 200 apparently healthy non-smoker males between 30-50 years selected by convenient sampling. Early morning samples of serum total testosterone and WBC count were obtained by phlebotomy after detailed medical history and thorough physical examination. All tests run on the same day and results were calculated.

Results: The mean (±SD) total testosterone was 15.92±6.32nmol/L. The frequency of low testosterone was 13.5%. TLC and neutrophils inversely correlated with testosterone ($p< 0.05$) whereas lymphocytes, eosinophils and monocytes did not correlate with testosterone.

Conclusion: Low testosterone is prevalent in Pakistani middle age non-smoker apparently healthy men. Significant inverse relationship of testosterone with WBC count showed that physiological variations in testosterone could modulate immune response in Pakistani men.

Key Words: Low testosterone, total leucocyte count, differential leucocyte count.

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INTRODUCTION

Testosterone in menis responsible for its effects commencing from the intrauterine life followed by male reproducibility and maintenance of spermatogenesis with the development of secondary sexual characteristics on reaching adolescence. Increased size of musculature with decreased fat mass, increased bone mass and cortical bone size and increased hematocrit as compared to females occurs after puberty under the influence of androgens. Interstitial cells of Leydig produce testosterone that reaches target tissues through blood. 2– 3% testosterone occurs in free form and the rest binds strongly with Sex Hormone Binding Globulin (SHBG) and loosely with albumin.[1]

[1.] Department of Physiology, Multan Medical & Dental College, Multan.
[2.] Department of Physiology, Islam Medical College, Sialkot.
[3.] Department of Physiology, Mohtarma Benazir Bhutto Shaheed Medical College, Mirpur, AJK.

Correspondence: Dr. Muhammad Omar Shamim, Associate Professor of Physiology, Islam Medical College, Sialkot
Contact No: 0333-2170847
Email: omar_shamim@yahoo.com

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The albumin bound and free form comprises bio available testosterone (20 – 40%)**.**

White blood cells (WBC) are involved in different body immune mechanisms based on exposure or non-exposure to antigens. These mechanisms include first line of defense in the form of direct killing of offending agent through phagocytosis by neutrophils, through the production of reactive oxygen species, release of antimicrobial peptides and expulsion of their nuclear contents to form neutrophil extracellular traps[2] and macrophages in the tissues. Response to exposure to antigens occurs through the activation of T cells and the production of antibodies by B cells. WBC play an important role in inflammatory conditions. Exposure to androgens alters immune cells functionality in autoimmunity and cancer.[3]Lymphoid and non-lymphoid cells of thymus and bone marrow express (AR) exhibiting development of B and T lymphocytes under the influence of androgen.[4]Testosterone acting directly through AR increases IL-10 production by $CD4^+$T lymphocytes.[5] Androgens exert an immune-suppressive role in males through decreased cytokine production[6] and suppress both humoral and cellular responses.[7] Testosterone decreases the overall activity of T cells and is more strongly associated with cytokine down-regulation in T-cell mediated response.[8] Testosterone decreases the number of B cells and antibodies. Cytokine BAFF (an essential survivor factor for B-cells) levels are higher in men with low testosterone.[9]Testosterone suppresses T-helper 1(Th 1)

differentiation by inhibiting IL-12 signaling in $CD4^+$ T cells through mediation of protein tyrosine phosphatase non-receptor type 1(ptpn1).[10]Conversely, immune response in females to estrogens is different and is associated with the probable development of autoimmune diseases.[11]

Sex hormones being the key regulators of immune responses effect the ability of mature immune cells.[12] Testosterone replacement therapy can decrease the levels of IgA, IgG and IgM antibodies and increased $CD8^+$ cells.[13] Androgen deficiency in men leads to increased levels of inflammatory biomarkers. Testosterone therapy attenuates inflammatory response in diseases like diabetes mellitus,[14]cardiovascular disease (CVD)[15] and rheumatoid arthritis.[16]

Considering the general immunosuppressive effect of testosterone and modulation of immunity, we tried to evaluate total leucocyte count (TLC) and differential leucocyte count (DLC) in relation to serum total testosterone in apparently healthy males aged 30-50 years.

MATERIALS AND METHODS

This was a cross sectional study conducted on apparently healthy male subjects aged 30-50 years. The study was conducted at Institute of Basic Medical Sciences (IBMS), Dow University of Health Sciences (DUHS) from 2010 to 2011. Smokers: due to variable levels of testosterone[17]and/or subjects doing regular heavy exercise; due to increased levels,[18] suffering from acute or chronic illnesses were excluded due to testosterone decline in acute and chronic illnesses,[19] total leucocyte count (TLC) more than 11×10^9 cells/L were excluded from the study. Individuals with history of testosterone supplementation, history of hypogonadism with signs and symptoms with evidence of low testosterone from medical records were not included. Sample size was 200 for the study.

Before sampling, participants signed a written consent approved by Institutional Review Board (IRB) of Dow University. Detailed medical history with general and systemic examination findings were noted down on prescribed proforma approved by IRB.

Early morning blood samples were obtained at Dow Diagnostic Reference Research Laboratories (DDRRL). Participants took a comfortable sleep and proper dinner to obtain maximum individual level of testosterone as hormone exhibits diurnal variation and missing meal leads to energy disturbances causing down regulation of male reproductive axis.[20]

2cc. blood was collected in purple top (containing EDTA) and yellow top bottles each for total leucocyte count (TLC) and serum total testosterone respectively.

TLC was estimated on hematology analyzer (Celltac α) using volumetric impedance method of cell counting. Testosterone levels were assayed on same day by Cobas e 411 analyzer with cut-off value of 9.70 nmol/L., upper and lower cut off for TLC was $\geq 11 \times 10^9$/l and $\leq 4 \times 10^9$/l respectively. SPSS v. 16.0 was used for statistical analysis. Threshold for statistical significance was set up at $p < 0.05$. t-test was applied for comparison of means. Pearson correlation was applied to see relationship between study variables.

RESULTS

The mean age ± SD of the participants of study was 38.72 ± 6.56 years. 68% respondents were married and 32% were unmarried. The mean serum total testosterone was 15.92 ± 6.32nmol/L. Mean TLC count was 7.56 ± 1.44. Mean neutrophil and lymphocyte percentages were 57.36 ± 8.38 and 47.52 ± 7.96 respectively. 27 participants had lower than normal testosterone (13.5%). The comparison of mean values between the two groups of testosterone was significant ($p<0.05$) for TLC and neutrophils but not for lymphocytes, basophils and monocytes (table I).

Table No.1: Mean values of study variables

Study variable	Mean ± S.D.
Age (years)	38.72 ± 6.56
Testosterone (nmol/L)	15.92 ± 6.32
Total Leucocyte Count ($\times10^9$ cells/L)	7.56 ± 1.44
Neutrophil %	57.36 ± 8.38
Lymphocyte %	37.52 ± 7.96
Monocyte %	3.06 ± 1.81
Eosinophil %	1.89 ± 0.99

Independent T test showed significant mean differences for the two groups of testosterone with TLC and neutrophils ($p<0.05$) but insignificant difference for lymphocytes, monocytes and eosinophils respectively ($p>0.05$). Pearson correlation showed that testosterone showed inverse relationship with TLC and neutrophil count ($p<0.05$). There was insignificant inverse relationship between testosterone and lymphocyte count ($p>0.05$). The correlation of testosterone with monocytes and eosinophils did not reach statistical significance ($p>0.05$) Table 2.

Table No.2: Correlation between serum total testosterone and WBC count

	Correlation coefficient (r)	p value
TLC	-.540	0.002*
Neutrophils	-.391	0.021*
Lymphocytes	-.580	0.532
Monocytes	-.040	0.571
Eosinophils	-.025	0.447

*Significant p value (<0.05)

DISCUSSION

In recent years, association of testosterone with inflammatory markers has been investigated including

WBC count as an indicator of inflammation.[21] Many studies looked for the associations of low testosterone with inflammatory markers in sample populations with obesity,[22]metabolic syndrome[23] and elderly males.[24]Our study aimed to look for the association of serum testosterone in relatively healthy males. Testosterone shows a decline with increasing age. On the other hand, immune response exhibits a change with aging as well.[25] Aging males show decreased testosterone and elevated CRP.[26] Higher levels of testosterone negatively correlate with WBC count [25] and CRP.[27]Low testosterone also showed significant association with elevated levels of pro-inflammatory cytokines TNFα, MIP1α and MIP1β.[28]Low levels of testosterone are associated with increased mortality associated with altered inflammatory states.[29]We tried to minimize the age effect by choosing the sample population between 30-50 years in our study. Mean testosterone levels in our study was15.92 ± 6.32nmol/l. which is consistent with population based study conducted in US by Tsilidiset al.[21] In our study, mean difference in two groups of testosterone was significant for TLC and neutrophils but not for mononuclear cells. Mean TLC was 7.56 ± 1.44, mean neutrophil percentage was 57.36 ± 8.38 and mean lymphocyte percentage was 37.52 ± 7.96 in our study, Tsilidis et al. reported mean TLC of 6.47 ± 0.13, mean neutrophil % of 58 ± 3.0 and mean lymphocyte percentage of 29.3 ± 0.3. The difference in lymphocyte percentage might be due to the broader age group in study by Tsilidis et al[21] while absence of any systemic or chronic illness in the participants of our study with comparatively narrower age group. Neutrophils activation requires pro-inflammatory cytokine TNFα. More recently, animal studies have showed that testosterone is responsible for higher recruitment of neutrophils in both testosterone dependent and independent tissues. Testosterone favors N2 like neutrophil phenotype expressing higher anti-inflammatory cytokines that may prove helpful in some non-bacterial type of inflammation and high expression of immunomodulatory molecules such as IL-10 and TGF-β1 that help in repairing tissues and resolving inflammation.[30]

Our study showed inverse relationship of total testosterone with TLC count ($p<0.05$). These findings are similar to the findings in a large population study conducted by Hering et al. comprising of 1344 men in which testosterone showed inverse relationship to TLC.[31] Another study conducted by Brand and colleagues showed inverse relationship of testosterone with TLC and neutrophils.[32] Our finding is also consistent with the findings of Park and Lee[33] who also reported inverse relationship of testosterone and SHBG with leucocyte count in Korean men aged more than 50 years.

Strength of the study was; it provided estimates of serum total testosterone in apparently healthy non-smoker sedentary males and its relationship with TLC and DLC. The apparently healthy sample population enabled us to minimize the cofounding factors for both WBC count and testosterone. Our study had some limitations: small sample size, convenient sampling, and inability to measure SHBG due to financial constraints.

CONCLUSION

Low testosterone is prevalent in Pakistani middle age non-smoker apparently healthy men. Significant inverse relationship of testosterone with WBC count showed that physiological variations in testosterone modulate immune response in Pakistani men.

Author's Contribution:

Concept & Design of Study:	Farooq Munfaet Ali Khan
Drafting:	Muhammad Omar Shamim, Rashid Mahmood
Data Analysis:	Shahzad Khan
Revisiting Critically:	Farooq Munfaet Ali Khan, Muhammad Omar Shamim
Final Approval of version:	Farooq Munfaet Ali Khan

Conflict of Interest: The study has no conflict of interest to declare by any author.

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