Alfarama Journal of Basic & Applied Sciences

Faculty of Science Port Said University

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http://sci.psu.edu.eg/en/

January 2025, Volume 6, Issue I

DOI:**https://doi.org/10.21608/AJBAS.2024.338944.1239**

ISSN 2682-275X

Submitted: 24/11/2024
Accepted: 15/12/2024

Pages: 66 - 74

The Role of Circulated Free Testosterone in Reducing Severity of Colorectal Cancer

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ABSTRACT

Background: It is still is still a great challenge to find strong and trustworthy colorectal cancer (CRC) prognostic biomarkers that can be linked to the likelihood of the disease's progression and severity. **Aim:** The purpose of this study was to assess the possible link between serum free testosterone (FT) and the development of CRC and its adverse outcomes in both male and female patients. **Results:** Despite female patients, results revealed that reduced FT levels (82.5 (2.1-121) pg/mL) were significantly (P<0.05) related to CRC cases compared to healthy controls (193 (164-206.5) pg/mL) and patients with benign polyps (150 (94.5-188) pg/mL). Serum FT had a good ability (AUC=0.852) to differentiate CRC cases from all non-cancer individuals. Serum FT levels (pg/mL) was significantly affected tumor aggressiveness in male patients including late stages (92 (75-113.3) vs. 130 (86.3-163.3); P=0.0002), lymph node invasion (104 (75.5-120) vs. 114.5 (81-155.3); P=0.0272), distant metastasis (95 (72-120) vs. 114 (81-144); P=0.0297), high grades (85 (72.8-118.5) vs. 128.5 (92-157.8); P=0.0002) and large size (97 (71.3-121) vs. 116 (102.5-150); P=0.0839). Serum FT was also significantly correlated with CEA (r =-0.227; P=0.049) in CRC male patients. **Conclusion:** serum FT hormone elevated levels appears to be protective against CRC development in male patients. Its reduced levels may be a reliable biomarker for monitoring CRC progression and may be helpful in preventing poor disease outcomes.

Keywords: CRC; Biomarker; Free testosterone; Severity; Poor outcomes

1. INTRODUCTION

Colorectal cancer (CRC), cancer of the large bowel, is the 3rd most common tumor globally [1]. Furthermore according the International Agency for Research on Cancer (IARC) estimates, it was the 2nd

most frequent cause of tumor-related death (after lung cancer) [2]. CRC is a diverse category of malignancies with a range of clinical and pathological manifestations [3]. The 5-year survival rate for colorectal cancer varies significantly among regions with different levels of economic development. For instance, the overall 5-year survival rate in the US is above 60%, but it is less than 40% in developing nations [4, 5].

Often, CRC is detected at late stages, limiting treatment options [6]. Despite improvements in CRC prevention and care, the prognosis for each patient varies greatly depending on CRC stage at initial diagnosis [3, 7]. Patient's survival is greatly affected by CRC stage as the 5-year survival rate in cases with CRC at a localized stage is as high as 90%, whereas this rate decreases to 70.4% for cases with regional invading and further decreases to 12.5% for cases with distant metastasis [8, 9]. Generally, the likelihood of survival increases with the early detection of CRC. The primary major goals for raising survival rates and quality of life are timely, suitable therapy, frequent follow-up, and early disease detection provision [10]. Furthermore, it becomes crucial to assess and look for alternate, best practices or very sensitive circulating markers to assess CRC proliferation with possible prognostic utility [11].

Testosterone (TEST) is the primarily male steroidal sex hormone that is generated in the males' testes. It plays a crucial role in male spermatogenesis, libido and secondary sexual characteristics development [12]. Clinical and experimental evidence associates TEST in prostate cancer aetiology [13]. Although these studies had limited power to evaluate the associations TEST and free TEST (FT) with prostate cancer aggressiveness, other studies found that low FT levels, rather than TEST, had important role in the cancer development, including prostate cancer aggressive determination [14]. Very limited studies reported that circulating levels of FT sex hormone binding globulin (SHBG) and TEST were inversely related to CRC risk [15].

The aim of this study was to evaluate serum levels of FT and their potential role in differentiating CRC from benign colorectal polyps and healthy controls. Furthermore, it was aimed to determine blood FT relation to poor CRC progression and aggressiveness including advanced stages, lymph node invasion, large tumor size, high histological grades, distant metastasis and elevated levels of established tumor markers carcinoembryonic antigen (CEA).

2. SUBJECTS AND METHODS

Study population

A total of 100 Egyptian patients (70 CRC and 30 benign colon polyps patients) comprised the study's cases They were undergoing a diagnostic colonoscopy at the Mansoura Oncology Centre at Mansoura University in Egypt for screening purposes or due to gastrointestinal problems. Benign disorders and CRC were diagnosed by colonoscopy and, on occasion, computed tomography. Additionally, 30 healthy individuals in general were included as study controls. CRC was categorised and staged using the worldwide Tumor-Node-Metastasis (TNM) [16]. There was no history of malignancy in either healthy controls or benign patients. This work, which adhered to the ethical criteria of the "Helsinki Declaration," was approved by Port Said University's scientific and ethical committees.

Laboratory tests

After the withdrawal of 10 mL fasting blood from each participant, serum samples were separated by centrifugation for 15 minutes (4500 rpm). Fresh serum specimens were tested for liver enzyme activity (alanine (ALT) and aspartate aminotransferase (AST)), as well as serum bilirubin, urea, creatinine, and albumin levels, using an automatic biochemistry analyser (Hitachi, Japan) and related commercial kits. Another portion of blood (treated with EDTA-K3) was used for a complete blood count using an automated analyser (Sysmex, Japan). Serum CEA was evaluated using commercial ELISA assay kits and in accordance with industry guidelines (MyBioSource, San Diego, USA).

Determination of serum free testosterone

The kits were supplied by their manufacturers, and an automated chemiluminescent immunoassay (CLIA; Maglumi 800, Snibe, Shenzhen, China) was used to assess total FT. Briefly, this is a competitive immunoluminometric assay. Use an anti-FT monoclonal antibody to label N-(aminobutyl)-N-(ethylisoluminol) (ABEI), and use a purified FT antigen to label fluorescein isothiocyanate (FITC). Control, calibrator or sample with FITC Label, ABEI label, and magnetic microbeads coated with anti-FITC are thoroughly mixed and incubated at 37℃, after sediment in a magnetic field and forming a sandwich, decant the supernatant, then cycle washing for one time. Thereafter, the starter reagents are added and a flash chemiluminescent reaction is performed. As relative light unit, light signal is assessed by a photomultiplier within three seconds and is proportional to specimen's FT concentration. Use an monoclonal antibody to label ABEI, and. Sample, Calibrator or Control with ABEI Label, displacing reagent, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37℃, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then wash it. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration (pg/mL) of FT present in samples.

3. STATISTICAL ANALYSIS

Appropriately, different parameters were expressed as mean±SD, median (interquartile range) or absolute numbers. All analyses were performed by SPSS vs 21 and GraphPad vs 9.0. To assess differences between cases and controls, student t-test, Kruskal-Wallis or ANOVA tests were used appropriately. $P<0.05$ is significant. Serum FT diagnostic ability was assessed using area under the receiver operating characteristic (ROC) curve. Correlation between FT and other parameters was evaluated by Pearson and Spearman correlation coefficients, appropriately.

4. RESULTS

Patients' characteristics

Cancer cases were matched by age (P =0.309) and gender (P =0.316) to healthy controls and cases of benign polyps. The clinical and haematological details of the cases are summarised in Table 1. The lack of a significant (P>0.05) difference in haematological, hepatic, and kidney-related variables between CRC cases and controls may have been related to the exclusion of any chronic diseases. CRC cases were substantially (P=0.035) correlated with high CEA values (Table 1). All CRC patients were categorised using the TNM staging system, tumour size, and degree of tumour differentiation (Table 1).

Reduced FT was associated with CRC

In contrast to women (Figure 1A), male patients (Figure 1B) showed a significant (P<0.05) correlation between CRC cases and lower serum FT levels (82.5 (2.1-121) pg/mL) when compared to healthy controls (193 (164-206.5) pg/mL) and patients with benign polyps (150 (94.5-188) pg/mL). The ability of decreased serum FT (AUC=0.852; 95%CI (0.784-0.921); Figure 2A) to distinguish CRC cases from all non-cancer persons was superior to that of CEA (AUC=0.751; 95%CI (0.638-0.864); Figure 2B).

Table 1. Characteristics of cases and controls

Variables	Colorectal cancer	Benign	Healthy	*P* value
Number	100	30	30	——
Gender (males/females)	71/29	19/11	20/10	0.316
Mean age ±SD, years	51.05±12.69	49.6±10.6	47.9±5.2	0.309
Hemoglobin (g/dL)	11.75±1.8	11.71±2.12	11.95±2.8	0.261
RBCs ($\times10^{12}$/L)	4.29±0.62	4.42±0.57	4.51±0.62	0.611
WBCs ($\times10^{9}$/L)	7.59±2.39	7.14±1.91	6.6±1.71	0.233
Platelet count ($\times10^{9}$/L)	273.12±52.12	265.1±65.4	282±59.7	0.173
ALT (U/L)	26.59±10.11	25.49±4.11	25.21±8.46	0.432
AST(U/L)	30.05±10.12	32.12±8.45	29.12±7.41	0.098
Bilirubin (mg/dL)	0.82±0.32	0.71±0.17	0.67±0.21	0.615
Albumin (g/dL)	3.73±0.82	3.92±0.46	4.1±0.36	0.124
Creatinine (mg/dL)	1.1±0.39	0.89±0.25	0.75±0.15	0.412
Urea (mg/dL)	30.6±9.2	29.8±6.1	25.6±4.89	0.621
CEA (U/L)	6.79 (2.1-15.4)	3.1 (2-6.5)	1.9 (1-3.37)	0.035
Tumor stage (Early/Late)	45/55	——	——	——
Lymph node (Negative/Positive)	62/38	——	——	——
Distant metastasis (Negative/Positive)	77/23	——	——	——
Histological grade (Low/High)	48/52	——	——	——
Tumor size (Small/Large)	40/60	——	——	——

Normally and non-normally distributed data were expressed as mean± SD and median (interquartile range), respectively. Early stage: T1-T2; Late stage: T3-T4; Low grade: (G1-G2); High grade: G3; Small tumor: ≤ 5 cm; Large tumor: >5 cm; RBC: red blood cell; WBC: white blood cell; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CEA: carcinoembryonic antigen. Significant differences were determined using *ANOVA* and *Kruskal-Wallis* test, appropriately. $P<0.05$ was significant.

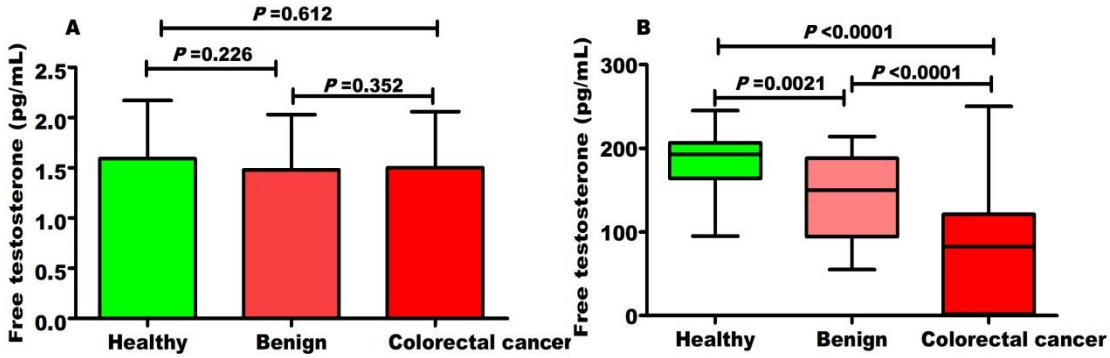

Figure 1. Serum FT and colorectal cancer development. Despite (A) female patients, reduced levels of FT were significantly related to (B) CRC male patients compared to cases with benign polyps and healthy individuals.

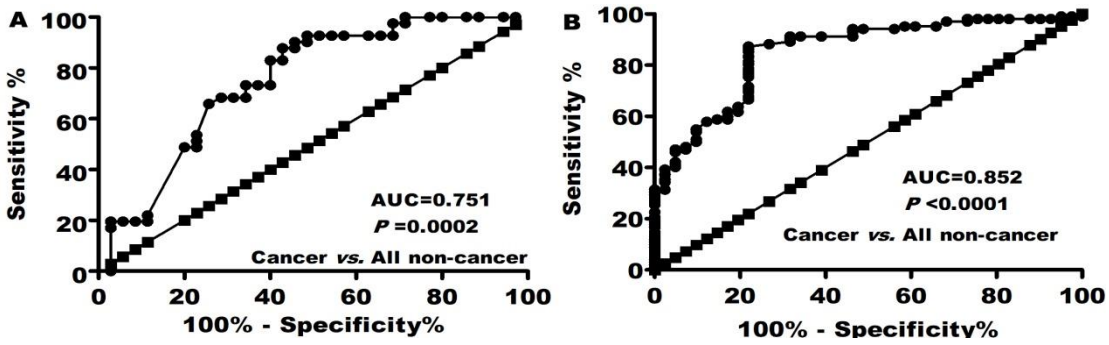

Figure 2. Compared to (A) CEA, ROC curve analysis revealed a superior diagnostic power of (B) FT in differentiating male CRC from all non-cancer individuals (benign and healthy combined).

Reduced FT was associated with tumor severity

Serum FT levels (ng/mL) significantly (P<0.05) influenced tumour aggressiveness in male patients in spite of female patients (Table 2), including late stages (92 (75-113.3) vs. 130 (86.3-163.3); P=0.0002), lymph node invasion (104 (75.5-120) vs. 114.5 (81-155.3); P=0.0272), distant metastasis (95 (72-120) vs. 114 (81-144); P=0.0297), high grades (85 (72.8-118.5) vs. 128.5 (92-157.8); P=0.0002), and large size (97 (71.3-121) vs. 116 (102.5-150); P=0.0839). FT was substantially linked with CEA (r =-0.227; P =0.049; Figure 3) in male CRC patients in addition to its considerable connection (P<0.05) with various tumour characteristics (Table 3).

Table 2. Association between FT levels and tumor severity in male compared to female patients. Data were expressed as median (inter quartile range) or mean±SD, appropriately.

Categories	Male patients		Female patients	
	Free testosterone (pg/mL)	***P* value**	**Free testosterone (pg/mL)**	***P* value**
Primary tumor stage				
Early stage (T1-T2)	130 (86.3-163.3)	0.0002	1.49±0.57	0.599
Late stage (T3-T4)	92 (75-113.3)		1.36±0.42	
Lymph node invasion				
Negative (N0)	114.5 (81-155.3)	0.0272	1.56±0.51	0.0529
Present (N1)	104 (75.5-120)		1.13±0.35	
Metastasis				
Negative (M0)	114 (81-144)	0.0297	1.45±0.51	0.3122
Present (M1)	95 (72-120)		1.17±0.35	
Tumor histological grade				
Low grade (G1-G2)	128.5 (92-157.8)	0.0002	1.56±0.57	0.1712
High grade (G3)	85 (72.8-118.5)		1.30±0.50	
Tumor size				
Small (≤ 5 cm)	116 (102.5-150)	0.0839	2.1±0.1	0.0779
Large (>5 cm)	97 (71.3-121)		1.23±0.6	

Table 3. Correlation between FT and tumor characteristics in male patients

Factor correlated with FT	Correlation coefficient (*r*)	*P* value
Tumor stage	-0.400	0.001
Tumor grade	-0.410	0.0001
Lymph node invasion	-0.204	0.049
Distant metastasis	-0.235	0.047
Tumor size	-0.292	0.048
CEA	-0.227	0.049

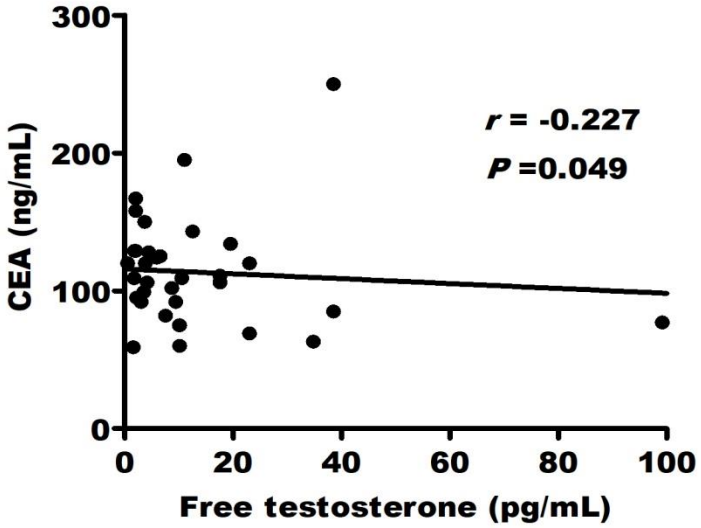

Figure 3. Correlation between serum FT and CEA serum levels in colorectal cancer male patients.

5. DISCUSSION

As the 3rd most common and 2nd leading cause of cancer-related deaths globally, CRC represent a massive challenge to the worldwide health care community [17]. About 20% of cases with CRC had metastatic disease at initial diagnosis, and their prognosis is dismal, with a five-year survival rate of 15.6% [17]. CRC stage at time of detection and early therapy initiation is what dictates survival rates and disease prognosis [3, 7]. Blood biomarkers' studying is not only making CRC clinical care better, but it is also revolutionising it [18]. They have revolutionized CRC management facilitating minimal residual disease (MRD) monitoring, personalized treatment, prevention and early detection [18]. This study aimed to evaluate serum FT potential relation with CRC development (compared to benign polyps and healthy controls) and also its association with the disease aggressiveness and poor outcomes in both male and female patients.

Our findings revealed that reduced serum FT was significantly (P<0.05) related to CRC (82.5 (2.1-121) pg/mL) compared to 193 (164-206.5) and 150 (94.5-188) pg/mL in healthy controls and benign polyps cases, respectively. It had a good superior diagnostic ability (AUC=0.852) compared to CEA (AUC=0.751) in diagnosing CRC. Furthermore, and despite female patients, serum FT reduced levels (pg/mL) in male patients was significantly (P<0.05) affected tumor aggressiveness including most tumor advanced features like late stages, lymph node invasion, distant metastasis, high grades and large size.

Besides its significant correlation with these tumor features, TEST was also significantly correlated with CEA (r =-0.227) in CRC male patients.

The role of sex hormones endogenous levels, including TEST, in CRC risk is unclear. However, preclinical data demonstrate a potential TEST role in CRC aetiology [19, 20]. Many reports have investigated the relation between subsequent CRC risk and endogenous sex hormone levels using prospectively collected blood samples [21, 22]. Furthermore, CRC risk has been reported to be higher in prostate cancer cases treated with androgen deprivation treatment [23, 24]. Taken together, the idea that sex hormones could contribute to the development of male CRC is supported by the body of research on the topic [15].

The results and findings of a recent dose–response meta-analysis provide suggestive evidence for the relation between TEST, FT and SHBG, and male CRC development. They found that circulating TEST and SHBG levels were inversely related to CRC risk. For FT, there was a non-significant inverse relation [15]. Other studies are in the line of this study and supporting our findings. Testosterone-albumin conjugates (TAC) are selective to membrane androgen receptors (mARs) and causes apoptosis through activation of caspase-3. When phosphorylated, Akt kinases stimulate CRC cells invasion and such kinases are dephosphorylated via mARs activation, thus decreasing CRC cell invasiveness and motility [19, 25]. In both experimentally-induced tumors in male rats and colonic wall, Izbicki et al. investigated hormonal manipulation effects on androgen receptor binding and on cancer development [26]. They found that androgens were reported to have an inhibitory effect on tumorigenesis; chemical castration elevated CRC incidence; and TEST administration generated a significant reduction in cancer development, particularly in the right colon, in surgically castrated rats [26]. Farahmandlou et al. performed an *in vitro* experiment to assess the association of TEST with human CRC cells (HT29) proliferation. Compared to control group, they reported that HT29 cells viability significantly decreased when exposed to TEST (1000 μg/mL) [27].

6. CONCLUSIONS

In this study the association between circulating FT levels and the aggressive behavior of CRC including late stage, high grades, large size and lymph node and distance invasion was evaluated. Interestingly, FT decreased levels were related to CRC severity and, thus, maintaining and controlling its level in the blood might be very important in preventing poor disease progression and outcomes. Future programs potential development for FT-based clinical control of CRC remains an open question for researchers.

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