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# Evaluation of Oxidative DNA Damage and Immunity Markers as Predicate of Prostate Patients

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**ABSTRACT**

Problems in health male accounts for 50% of all occurrences of infertility and is the only cause of 10–30% of cases. Male-specific malignancies and reproductive problems have become a major public health concern due to their sharp rise in recent years.

**Study Aim:** In this research, the levels of some different biomarkers will be investigated –Prostatic Acid Phosphatase (PAP), Prostate Cancer Gene 3 (PCA3), reactive oxygen species 8-hydroxy-2'-deoxyguanosine (8-OHDG), and immunological cytokines including IL-18 and prostate specific antigen (PSA) at the fertility center as a diagnostic marker of hyperplasis between patients who were infertile due to prostate cancer, patients who had prostatic hyperplasia, and healthy individuals who were fertile controls.

**Material and methods:** The study involved 100 males, choose 80 divided into 58 prostate hyperplasia male infertile, 10 fertile control male and 22 patients' prostatic cancer infertile visit the fertility center and Doctor's Consulting Clinic in peroid between 1/11/2024 to 1/3/2025.

**Results:** Significant differences in biomarker levels were observed across the groups. The levels of IL-18, PSA, PAP, and PCA3 were also considerably higher in patients with hyperplasia and cancer when compared to the levels seen in controls (The p-value for PAP was less than 0.01, the p-value for PCA3 was less than 0.002, and the p-value for IL-18 was less than 0.001). Notably, 8-OHDG levels were significantly lower in hyperplasia and cancer groups compared to controls ($p < 0.05$). While mean values for PCA3 and IL-18 were higher in cancer than hyperplasia, these differences did not reach statistical significance also, PAP demonstrated clear differences between all three groups and 8-OHDG was significantly lower in disease groups compared to controls.

**Conclusion:** These results suggest that, 8-OHDG, PAP, PCA3, PSA, and IL-18 may serve as valuable biomarkers for differentiating between healthy individuals, those with prostatic hyperplasia, and those with prostate cancer.

**Recommendation:** These biomarkers have potential clinical utility in distinguishing prostate health and disease states, conduct more in-depth studies to explore the relationship between BMI and P. Hyperplasia/Cancer 8-OHDG, PAP, PCA3, PSA, and IL-18 and investigate potential mechanisms, such as hormonal influences, inflammatory markers, and metabolic factors.

***Keywords:*** *Predicator markers, oxygen species, Prostate cancer, cytokines.*

## 1. INTRODUCTION

Over the last several years, the prevalence of male-specific reproductive illnesses and cancers has dramatically increased, which positions them as a serious public health concern. Prostate cancer, often known as PC, is the kind of cancer that is diagnosed in males the most frequently and is associated with one of the highest rates of death due to cancer. Although the precise mechanisms responsible for PC have not been determined, both epigenetic and genetic changes have a role in the onset and advancement of the illness. Another complicated and little-understood issue that is thought to affect a significant number of males is male infertility. Several theories have been put forward, including Y chromosome alterations, chromosomal abnormalities, and impaired DNA repair processes. The connection between prostate cancer and infertility is becoming acknowledged (1).

The amount of data linking male infertility to an increased risk of carcinogenesis is increasing. Most research has focused on two types of prostate cancer: Prostate cancer (PC) and Testicular cancer (TCa) (2). Many exogenous and environmental variables associated with the progression of PCA3 or considered etiologically significant for the transition from latent to clinically relevant PCA3, are now included in the guidelines issued by the European Urological Associations (EAU) and American Urological Associations (AUA). On the other hand, none of these recommendations address the association between prostate cancer risk and men's reproductive health or the quality of their semen. Additionally, the cancer screening practice recommendations only consider family history, age, and African heritage (3; 4; 5; 6). In addition to evaluating spermatogenic abnormalities and prostate cancer indicators, this study investigates the link between prostate cancer and male infertility and pinpoints the underlying causes, risk factors, and biological mechanisms involved.

## 2. MATERIALS AND METHODS

All of the blood samples and the examination of the semen fluid were taken using a conventional method at the Al-Sadr Teaching Hospital in Najaf, Iraq, during the period from 1/11/2024 to 1/3/2025. In this clinical investigation, a total of 100 males were screened, and 80 of them were selected into three groups: 58 men with prostate hyperplasia who were infertile, 10 men who were fertile controls, and 22 men who had prostate cancer infertile that visit the fertility centre and Doctor's Consulting Clinic Samples were obtained prior to surgery in order to measure the enzymatic activity of both fertile males as a control group and those with prostate tumors and hyperplasia. This study included three groups of participants aged 40 to 75 years: (i) 22 patients with infertility due to prostate cancer; (ii) 58 patients with infertility due to benign prostatic hyperplasia; and (iii) 10 healthy fertile men serving as the control group. Standard equation for calculating BMI is BMI ($kg/m^2$) = weight (kg) / height ($m^2$). Without the use of anticoagulant, blood was drawn into a serum separator vacutainer and left to coagulate at room temperature for twenty to thirty minutes. After centrifugation was used to separate the sera, all the specimens were immediately aliquoted and kept at -70°C until they were processed in batches using deep freezing.

### Method Description

Tests were conducted using ELISA method, specifically the Automated Chemiluminescent Immunometric Assay. The patient's samples were introduced to a solid phase coated with a mouse monoclonal antibody specific for PAP, PCA3, PSA, 8-OHDG, and IL-18. To create an antibody sandwich complex, a goat-anti-PAP, PCA3, PSA, 8-OHDG, and IL-18 alkaline phosphatase conjugate is incorporated. Washing was performed to remove excess conjugate, followed by the addition of an adamantyl dioxetane phosphate substrate to facilitate chemiluminescence. The light emission correlated with the concentration of PAP in the specimen.

### Seminal fluid analysis

Semen collection was performed by masturbation following a 3–5-day period of abstinence, along with the sample being placed straight onto a Petri dish that is clean, dry, and sterile, and then being placed in a location that is both private and quiet, which is next to the laboratory that analyzes sperm. The subject's name, identification number, and collection date and time should be correctly labeled on the container. To help in the liquefaction process, the specimens were placed in an incubator set at the temperature of 37 degrees for thirty minutes. The liquid semen is combined using a glass Pasteur pipette for several seconds, following which the sample is meticulously analyzed using microscopic and macroscopic techniques within one hour after collection, in line with the WHO manual (8).

### Statistical Analysis

For all of the statistical studies, the usage of SPSS version 28.0 (SPSS Inc., Chicago, Illinois, United States) was used. For the purpose of comparing clinical data in the form of continuous variables, analysis of covariance was used. These variables were reported as the median and interquartile range (IQR) for skewed data, or as the mean plus or minus the standard deviation (SD) for data that was normally distributed. For the purpose of comparing categorical variables, which are reported as percentages, Fisher exact or chi-square tests were used. A Pearson's or Spearman's rank correlation analysis was used to evaluate the connections between markers and each and every demographic variable. Additionally, multiple logistic regression was used to investigate the independent predictors of prostate cancer. Statistical significance was determined to be achieved when the p value was less than 0.05, and all statistical tests were conducted using a two-sided design [7].

## 3. RESULTS AND DISCUSSION

### Demographic categories:

The study involved 100 males, choose 80 divided into 58 prostate hyperplasia male, 10 fertile control male and 22 patients' prostatic cancer, Although the control group's mean age is slightly greater, $p = 0.637$ indicates that the difference isn't statistically significant. The age distribution (below and above 40 years) also shows no significant differences between the groups ($p = 0.407$) Prostate Hyperplasia Prostate Cancer and controls fertile male as showed results in table (1) compares the two groups across several demographic and health control categories. Despite the lack of statistical significance in age differences, age may serve as a confounding variable in numerous health conditions. In the case of benign prostatic

hyperplasia, for example, which is a non-cancerous condition that causes the prostate to expand and mostly affects men over the age of fifty, the quality and harmony of patients' lives are significantly diminished as a result (8). Pollutants, environmental, social, psychological, genetic, and dietary aspects that distinguish urbanites from rural residents are only a few of the many important distinctions between the two. However, men's mental health may be impacted by the demands and stresses of their jobs. Additionally, variables related to marital and home dynamics contribute to a worsening of the oxidant/antioxidant status issue (9).

The mean BMI is notably higher in the control group, but the t-test reveals no statistically significant difference (p = 0.701). However, the categorical analysis of BMI (<25 and >25 kg/m^2) shows a significant difference (p = 0.009). The control group has a much higher percentage of individuals with a BMI greater than 25 kg/m^2, table (1). The significant difference in BMI distribution is a crucial finding. It suggests that higher BMI (overweight/obesity) may be a protective factor or at least associated with the control group (fertile males), and lower BMI is associated with the patient group (P. Hyperplasia/Cancer). A number of indicators of risk for the onset of prostate cancer and hyperplasia have been identified via the use of scientific data derived from clinical trials. The development of age, an increase in weight, a lack of adequate physical activity, dietary habits, a family history of obesity, and overall being overweight or obese are all factors that contribute to obesity (10). On the other hand, insulin resistance, inflammatory processes, and dysfunction of adipose tissue may all have a role in the development of prostate cancer, even if the precise mechanisms that underlie these relationships are not fully known (11).

**Table (1): Demographics Features in Prostate Hyperplasia Prostate Cancer and Controls fertile male**

| **Demographics Categories** | | **Patients N=80 (88.9%)** | | **Control N=10 (11.1)** | | **Chi-Square** | **p-value** |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | | |
| **Age (year)** | Mean ± SD | 43.16±11.25 | | 45.00±14.17 | | | 0.637# NS |
| | Less than 40 | 35 | 43.8% | 3 | 30.0% | 0.69 | 0.407 NS |
| | More than 40 | 45 | 56.3% | 7 | 70.0% | | |
| **BMI (kg/m^2)** | Mean ± SD | 22.57±3.27 | | 26.4±2.59 | | | 0.701# NS |
| | Less than 25 | 52 | 65.0% | 2 | 20.0% | 7.50 | 0.009* |
| | More than 25 | 28 | 35.0% | 8 | 80.0% | | |

Significant differences at p-value ≤0.05*. #: independent t-test between two continuous variables.

**Table (2): Assessment of Predicate Biomarker Levels in Prostate Hyperplasia, Prostate Cancer, and Control Groups**

| Studied Groups | | | | Percentiles | | p-value |
|---|---|---|---|---|---|---|
| | | Mean | SD | Median | IQR (Q1-Q3) | |
| PAP (pg/ml) | P. Hyperplasia | 405.9 | 112.0 | 394.2 | 317.5 -505.4 | G1 vs. G2=0.008<br>G1 vs G3=0.001<br>G2 vs. G3=0.001 |
| | P. Cancer | 538.0 | 177.0 | 565.0 | 383.4 -670.5 | |
| | Control | 168.3 | 38.1 | 164.7 | 135.4 -206.2 | |
| PCA3 (pg/ml) | P. Hyperplasia | 4.6 | 1.1 | 4.7 | 3.8 -5.5 | G1 vs. G2=0.097<br>G1 vs G3=0.002<br>G2 vs. G3=0.001 |
| | P. Cancer | 7.0 | 1.7 | 6.8 | 5.5 -8.7 | |
| | Control | 2.2 | 0.8 | 2.1 | 1.5 -2.9 | |
| 8-OHDG (pg/ml) | P. Hyperplasia | 195.2 | 40.9 | 195.3 | 162.2 -219.9 | G1 vs. G2=0.012<br>G1 vs G3=0.005 |
| | P. Cancer | 172.9 | 42.1 | 169.1 | 137.7 -186.7 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Control | 355.3 | 99.4 | 350.3 | 259.1 -468.6 | G2 vs. G3=0.001 |
| IL-18 (ng/ml) | P. Hyperplasia | 12.5 | 1.8 | 12.5 | 11.4 -13.5 | G1 vs. G2=0.511 |
| | P. Cancer | 13.3 | 3.0 | 13.2 | 10.8 -15.1 | G1 vs G3=0.001 |
| | Control | 6.2 | 2.4 | 5.2 | 4.7 -8.0 | G2 vs. G3=0.001 |

G1 = P. Hyperplasia, G2 = P. Cancer , G3 = Control. Significant differences at p-value ≤0.05*. #: independent t-test between two continuous variables.

Table (2) shows that compared to the Control group, PAP levels are considerably higher in both prostatic hyperplasia and prostate cancer ($p < 0.001$). Prostate Cancer shows the highest PAP levels, significantly higher than prostate Hyperplasia ($p = 0.008$). PCA3 Levels are significantly higher in prostate Cancer than in both P. Hyperplasia ($p = 0.097$, approaching significance) and Control ($p = 0.001$) P. Hyperplasia also shows significantly higher PCA3 compared to the Control ($p = 0.002$) 8-OHDG Interestingly, the Control group has significantly higher 8-OHDG levels compared to both prostate Hyperplasia and prostate Cancer ($p < 0.005$) prostate Hyperplasia had a significantly higher 8-OHDG level than the P. Cancer group. IL-18 levels are significantly higher in both prostate Hyperplasia and prostate Cancer compared to the Control group ($p = 0.001$). The IL-18 levels in the prostate cancer and prostate hyperplasia groups are comparable ($p = 0.511$). Benign and malignant illnesses of the prostate are the most prevalent diseases affecting males in industrialized or often war-torn nations. As the most common kind of cancer and one of the most deadly detected abnormalities, PC ranks third behind lung and colorectal malignancies and raises male mortality. Prostate hyperplasia, on the other hand, is a non-malignant abnormality that causes the prostate to enlarge and inhibits fertility beyond the age of 45. As a result, it has a significant impact on the material and psychological well-being of patients (8).

The significant elevation of PAP and PCA3 in prostate Hyperplasia and prostate Cancer suggests their potential as diagnostic markers for these conditions. PCA3 shows strong discriminatory power. Also, the higher levels of these markers in the prostate Cancer group shows that they may also be used to distinguish between hyperplasia and cancer. further investigation. 8-OHDG is a marker of oxidative DNA damage, and its elevation in healthy controls is counterintuitive. This could indicate a unique physiological state in the control group or potential confounding factors. The lower levels in the cancer group may show that the cancer cells have a higher capacity for DNA repair, or other factors. Recent studies have identified various pathologic and serologic biomarkers that exhibit greater precision compared to serum prostate specific antigen (PSA), thereby reducing unnecessary biopsies and informing treatment decisions (12). PCA3 is one of the most useful biomarkers in the identification of prostate cancer, among those that are available (13). The fact that PCA3 gene expression differs between PCa and noncancerous tissues greatly aids clinicians in differentiating PCa from other prostatic disorders (14). Located on the long arm of chromosome nine, the PCA3 gene is 23 kilobytes long and contains 4 exons and nucleic acid. In normal cells, the PCA3 gene is unable to be translated into protein (15). This method effectively distinguishes between benign and cancerous prostate cells with an accuracy nearing 100%, as it targets a specific biomarker that is overexpressed in more than 90% of prostate cancer cells (16).

Prostate Hyperplasia and prostate cancer are associated with increased IL-18 levels, it indicates that inflammation is a contributing factor in both conditions. The rise in IL-18, a pro-inflammatory cytokine, raises the possibility that it contributes to pathogenesis. It seems that IL-18 may not be a viable marker for differentiating between prostatic hyperplasia and prostate cancer, because the levels are identical across the two groups. The complicated etiology of obesity, which includes dietary choices, levels of physical activity, and hereditary factors interacting, may lead to a raised IL-level. Social, environmental, economic, and behavioral variables all play a role in this etiology, and they are strongly linked to insulin resistance and the cancer it causes (17). A direct relationship exists between the polymorphism of the TNF-α gene's promoter and the anti-inflammatory and pro-inflammatory responses. This polymorphism has an effect on the production of TNF-α, which in turn leads to variations within the immune response of individuals and has an impact on the likelihood that they may develop prostate cancer. Consequently, it is possible to consider it an additional biomarker in addition to PSA, which has the potential to represent the activity of both PCa and BPH (13). The biological activity of the cytokine IFN-γ is often linked to antitumor and cytostatic/cytotoxic processes during a cell-mediated adaptive immune response, as well as its crucial function in coordinating humoral immune responses (18). In individuals suffering from benign prostatic hyperplasia (BPH) or prostate cancer (PCa), increased levels of IFN-γ in the blood might indicate an adaptive response to the role of lymphocytes in tumor management and development. The presence of IFN-γ receptors is linked to an increased risk of tumor development. To improve the immune system's ability to recognize these changed cells, IFN mainly acts on the changed cell itself. Furthermore, it demonstrates several characteristics, including procarcinogenic, cytostatic, and cytotoxic effects under certain conditions (19).

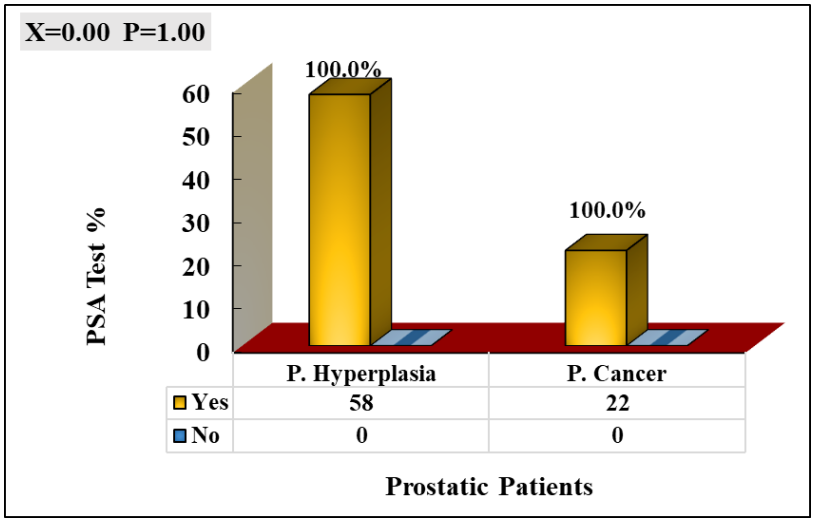


**Figure (1): Agraph illustrating the percentage of patients with Prostatic Hyperplasia and Prostatic Cancer PSA test.**

The results show 100% PSA (Prostate-Specific Antigen) figure (1) the most striking result is that every patient in both the prostate Hyperplasia and prostate Cancer groups received a PSA test. In terms of PSA testing, the statistical analysis reveals that there is no statistically significant difference between the two groups when compared in fertile is normal (Chi-square = 0.00, p = 1.00). About of 100% testing rate suggests that PSA testing is a standard practice for both prostate Hyperplasia and prostate Cancer patients in this particular site. This could reflect established clinical guidelines or local protocols. New biomarkers are required in cancer to improve and control PCA patient-specific decision-making, treatment, and therapy monitoring, according to all current research. Thus, it is possible that a number of growth factors, cytokines, and other proteins may need to be systematically up-regulated in reaction to cancer's development and therapy (12). In addition, since cancer patients might have a wide range of reactions to treatment, it is not possible to utilize a single biomarker like PSA to represent the true tumor mass while monitoring cancer, particularly in patients undergoing chemotherapy and radiation. This is why it could be crucial to use a new clinical diagnostic to detect tumors and assess how treated normal tissue reacts (20).

## 4. CONCLUSION

The result indicated the levels alternative of PAP and PCA3 are promising diagnostic markers for prostate Hyperplasia and prostate Cancer, with PCA3 showing strong discriminatory power in compensations of detected levels of cytokine IL-18 highlights the inflammatory aspect of these conditions, all patients with both prostate Hyperplasia and prostate Cancer underwent PSA testing, indicating a consistent and high rate of PSA utilization in these patient groups. No statistically significant difference in PSA testing % was found between the hyperplasis and cancer groups, according to the data.

## 5. RECOMMENDATIONS

Investigate the underlying mechanisms contributing to the observed biomarker changes. Specifically, investigate effect these markers and BMI in the 8-OHDG levels . also an analysis of the cost-effectiveness of this high rate of PSA testing could be valuable, especially considering the potential for over diagnosis and overtreatment associated with PSA screening.

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