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Pleuro-Pericardial Inflammation and Effusion: A Rare Acute Initial Presentation of Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis is a systemic inflammatory arthritis characterized by joint pain and morning stiffness. The affected joints are typically symmetrically affected, and given the inflammatory nature of this condition, patients often present with warmth and erythema around affected joints as well as fatigue. Extra-articular manifestations, especially pleuro-pericardial inflammation, are rare initial presentations, although may be seen in advanced or undertreated disease. Case Presentation: We describe a case of a rheumatoid arthritis presenting atypically in a middle-aged male who came to the emergency department complaining of diffuse muscle pain and swelling in the distal extremities. Cardiac ultrasound revealed pleuro-pericardial inflammation and effusions. Myositis and infectious causes were ruled out and bilateral hand x-rays did not show erosions or any evidence of arthritic changes. All rheumatological auto-antibodies were negative except for rheumatoid factor and anti-cyclic citrullinated peptide (CCP) and a diagnosis of rheumatoid arthritis was made. The patient was started on prednisone with excellent response. Conclusions: This case highlights that rheumatoid arthritis can uncommonly present initially with extra-articular manifestations that are often manifested in advanced disease. Typically, extra-articular manifestations, especially those as severe as this patient's, occur with untreated, advanced disease and could accompany extensive arthritic joint changes. Thus, it is important to have an understanding of rare, atypical presentations of rheumatoid arthritis so that a high index of suspicion can be maintained to make the diagnosis and initiate treatment in a timely manner.

Keywords

Rheumatoid Arthritis, Atypical Rheumatoid Arthritis, Pleuro-Pericardial

Inflammation, Pleuro-Pericardial Effusion

1. Background

Rheumatoid arthritis (RA) is the most common systemic inflammatory arthritis. RA is an autoimmune condition where immune cells target specific tissue, often in joints, causing patients to present with symmetric pain and stiffness in multiple joints, inflammation, and fatigue. RA most often affects women, smokers, and those with a family history of the disease. The lifetime prevalence of RA is 1% globally. RA typically presents between the ages of 30 and 50 years old with symmetric pain and stiffness in multiple joints and morning stiffness lasting more than one hour. The wrist, metacarpophalangeal, and proximal interphalangeal joints are most commonly affected while distal interphalangeal and lumbar spine joints are not usually affected. Systemic symptoms including fatigue, weight loss, and anemia may also be present [1]. Diagnosis can be made with consistent symptom presentation as well as positive serology for rheumatoid factor and anti-citrullinated protein antibody. Extra-articular symptoms that affect other bodily systems can develop as well, however, typically, patients present initially with articular symptoms before severe systemic manifestations develop.

Without proper treatment, RA can progress to involve other systems, including pulmonary and cardiovascular systems. These are known as extra-articular manifestations (EAM) and they usually occur later in the course of disease as a result of undertreatment or treatment resistance. The most common EAM is rheumatoid nodules, affecting about 30% of patients [2]. One such EAM is Felty Syndrome, characterized by RA, neutropenia, and splenomegaly [3]. Caplan Syndrome, also known as rheumatoid pneumoconiosis, is another EAM, characterized by an inflammatory lung condition in reaction to exposure to coal, asbestos, and/or silica, in patients with diagnosed RA [4]. Rarely, severe pleuro-pericardial inflammation and effusion may occur early in the course of disease as an initial presentation. It is rare for patients to present initially with extra-articular manifestations of rheumatoid arthritis that, in these cases, making the proper diagnosis is difficult and often delayed. These cases are usually published in the literature to highlight the possibility of these atypical presentations. However, to our knowledge, there are no published cases in the literature that are identical to the case presented here. This case presentation describes an atypical initial presentation of rheumatoid arthritis in the form of severe pleuro-pericardial inflammation and effusion with other non-specific symptoms in the absence of arthritic joint changes.

2. Case Presentation

The patient is a 45-year-old male who presented to the emergency department

with approximately two weeks of worsening generalized muscle pain, cramping, and diffuse swelling in the distal extremities. The patient's only past medical history was a three-month hospitalization when he was an 8-year-old child for muscle and joint pain that began after moving to a new state. He was treated for allergies, although the true cause was never determined. He continued to deteriorate at that time, and the patient's family moved again to a different state where the patient's symptoms spontaneously resolved. He never took any medications long term, and was not taking any medications at presentation. Two weeks prior to the patient's initial presentation, he felt extreme cramping and proximal muscle weakness after spending an afternoon mowing his lawn. He then noticed a rash appeared on his medial ankles and shins, as well as the dorsal aspects of his hands bilaterally. He wrapped his extremities in ice packs, took ibuprofen, and the pain resolved by the next day. One week later, the patient took a flight to Toronto for a vacation and endorsed heavy alcohol consumption. His muscle pain and weakness returned during the return flight and progressively worsened over the following four days despite consistent ibuprofen use. During this time, distal extremity swelling appeared, and the patient was no longer able to cope at home and presented to the emergency department.

Musculoskeletal examination revealed profound proximal muscle weakness and pain in the bilateral upper and lower extremities. Diffuse swelling and secondary skin tightening was visible in the dorsal aspects of the bilateral hands and feet not limited to the wrist, metacarpal and phalangeal joints. Skin examination revealed erythematous papules over the metacarpophalangeal joints bilaterally and the proximal interphalangeal joints on the left. At the emergency department, an erythematous rash was seen at the periorbital area along with flushing in the anterior and posterior aspects of the neck. Erythema was also present over the extensor surfaces of the bilateral elbows and knees and a maculopapular, erythematous rash was appreciated at the medial aspects of the bilateral ankles and shins.

Given the proximal distribution of acute-onset muscle pain and weakness coupled with the cutaneous findings potentially consistent with a heliotrope rash and gottron papules, dermatomyositis was high on the list of differential diagnoses, but creatine kinase was normal. Given the recent travel history and significant amount of time spent outdoors in his yard and garden, infectious causes were highly considered. Inflammatory and infectious markers were measured as shown in **Table 1** and **Table 2**. Arthrocentesis was performed on the right knee but gram stain and cultures were negative. A panel of rheumatologic autoantibodies was also sent early in the hospital course due to a high index of suspicion of rheumatological disease in the setting of multiple confounding variables in his presentation.

A focused cardiac ultrasound, shown in **Figure 1**, was then performed since he continued to deteriorate and developed mild, vague chest achiness. It revealed extensive pericardial inflammation and effusion including left-sided pleural

Table 1. Values for inflammatory markers for each day of the hospital stay (NR: normal range).

	Day 1	Day 2	Day 3	Day 4	Day 5
Creatine Kinase (U/L) NR: 39 - 308	68	72	35		
C-Reactive Protein (mg/L) NR: 0 - 8	199.6	177.5	147.4	145.8	61.2
Sedimentation Rate (mm/hr) NR: 0 - 14	83	72	70	99	75
White Blood Cell Count (count $*10^9$/L) NR: 3.5 - 10	13.8	13.0	12.6	13.6	14.3

Inflammatory markers measured and tracked throughout the 5-day hospital stay.

Table 2. Values for rheumatologic autoantibodies and infectious markers sent (NR: normal range).

	Day 1	Day 2	Day 3
Anti-nuclear antibody (NR: <1:40)	<1:40		
Anti-dsDNA (IU/mL) (NR: <4)	1		
Anti-Jo (AI) (NR: 0 - 0.9)	<0.2		
Anti-RNP (AI) (NR: 0 - 0.9)	<0.2		
Anti-Scl70 (AI) (NR: 0 - 0.9)	<0.2		
Anti-Smith (AI) (NR: 0 - 0.9)	<0.2		
Anti-SSA (AI) (NR: 0 - 0.9)	<0.2		
Anti-SSB (AI) (NR: 0 - 0.9)	<0.2		
Rheumatoid Factor (IU/mL) (NR: <13)		46.7	
Lyme Reflex (NR: <0.9)		0.19	
Anti-CCP (U/mL) (NR: 0 - 2.9)			>300.0
Myeloperoxidase antibody (AI) (NR: 0 - 0.9)			<0.2
Proteinase 3 antibody (AI) (NR: 0 - 0.9)			<0.2
Quantiferon TB values (2) (IU/mL)			−0.02
Anti-streptolysin O (IU/mL) (NR: <201)			28
B. Burgdorferi PCR			Negative
B. Garinii/D. Afzelii PCR			Negative
B. Mayonii PCR			Negative

Rheumatologic autoantibodies and infectious markers measured on days 1 - 3 of the hospital stay with abnormal values indicated in red.

effusions. Interestingly, the patient denied shortness of breath, and never required supplemental oxygen. By this time, all infectious and most rheumatological auto-antibodies were negative thus far except for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP), which were pending. Rheumatology was consulted and the patient was started on prednisone for symptom control, which resulted in significant relief. Colchicine was never required to treat the pericarditis. Finally, RF and anti-CCP came back positive, and a final diagnosis of rheumatoid arthritis was made. Bilateral hand x-rays were obtained, shown in Figure 2, which did not reveal any erosions or evidence of arthritic changes, suggestive of an atypical acute presentation.

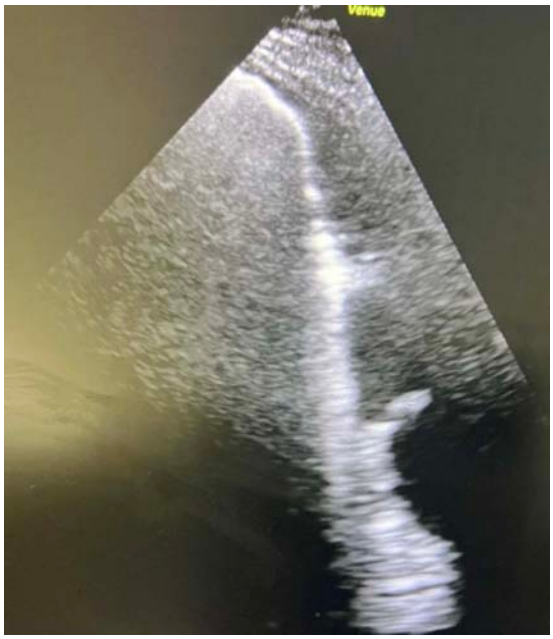
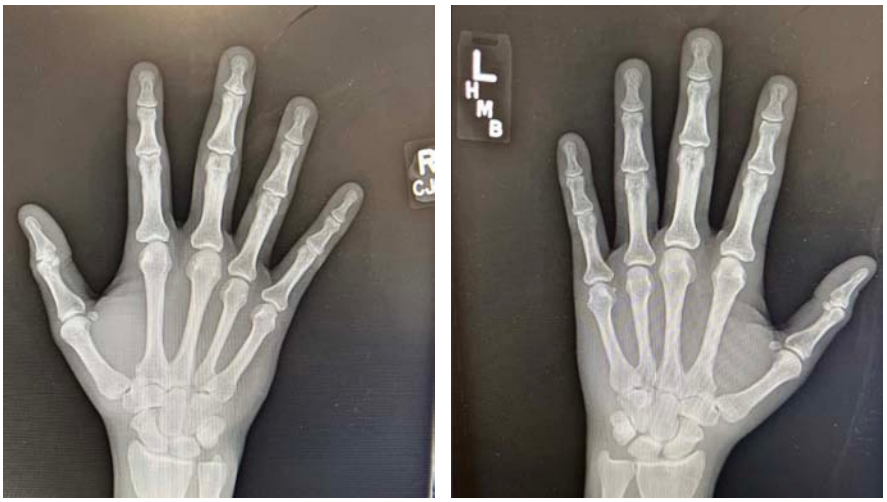


Figure 1. Cardiac ultrasound.



(a) (b)

Figure 2. (a): Right hand X-ray; (b): Left hand X-ray.

The patient was started on 40 mg prednisone as well as oxycodone and morphine for pain management in the hospital, which resulted in significant improvement. He was discharged on 40 mg of prednisone and advised to follow-up with rheumatology as an outpatient and his primary care provider. One week later, he was seen by rheumatology and all of his symptoms had significantly improved. At this time, he was started on methotrexate 15 mg daily and folic acid and advised to avoid alcohol use. The patient was seen again one month later and was doing well without functional limitations.

3. Discussion

Rheumatoid arthritis (RA) is the most commonly diagnosed systemic inflammatory arthritis. Without sufficient treatment, EAM affecting other bodily systems may develop. Given that the disease process is usually quite advanced when extra-articular manifestations develop, it is very unusual that these would be part of an initial RA presentation in the absence of joint changes suggestive of an inflammatory arthritis. EAM are most frequently seen in patients with severe disease and are associated with increased mortality [5]. The major risk factors that are clinical predictors of the development of EAM include male gender, smoking, advanced joint disease, low level of functioning, and very elevated inflammatory marker levels [2]. Clinicians must follow patients with these risk factors extremely closely to ensure their disease remains under control.

Given that the primary characteristic of RA is joint inflammation resulting in joint damage especially in chronic settings, it is unlikely that EAM occurs as part of an initial, severe presentation in the absence of any joint changes [5]. Our question of whether this could be a missed insidious late presentation was answered by the normal bilateral hand x-rays that did not show erosions, suggesting that this case was an acute onset of disease rather than a chronic ongoing process. Although the patient's alcohol use preceded the onset of disease, the literature on the association of alcohol and risk of RA is inconclusive with mixed evidence of increased, decreased and in some studies showing no association [6] [7] [8]. Furthermore, only few cases of RA initially presenting with pleural and/or pericardial effusion have been reported, however, of those that have, all patients complained of chest pain and/or shortness of breath [9] [10] [11] [12]. Our patient denied both these symptoms, making this presentation even more unusual.

According to the American College of Rheumatology, methotrexate continues to be recommended as a first-line agent for treatment of RA. The literature suggests that treatment recommendations do not vary depending on the presence or absence of EAM. During acute flares of the condition, steroids such as prednisone can be added to the treatment regimen. For patients that cannot tolerate methotrexate or have a risk factor for hepatotoxicity, other medications, such as disease-modifying antirheumatic drugs (DMARDS) can be used. These medications include, but are not limited to, leflunomide, hydroxychloroquine, and sul-

fasalazine [1]. The appropriate treatment regimen may vary between patients so it is important to consider medical history, risk factors, and side effect profiles in determining the optimal regimen. When EAM of RA are present, a more aggressive therapeutic approach is taken because EAM are typically associated with a far more advanced and severe disease state, which increases morbidity and mortality in these patients. Additionally, worse long term outcomes associated with EAM leave patients susceptible to functional impairment depending on the complications they experience. However, since this is an initial presentation of RA with EAM, the patient described here responded well on a standard prednisone and methotrexate regimen without the need for an aggressive treatment approach.

4. Conclusion

This case highlights how RA can rarely present initially with predominantly extra-articular manifestations and non-specific musculoskeletal symptoms. Given that many organ systems can be affected by EAM, it is important that RA be considered as a possible diagnosis when an inflammatory process is evident and cannot be explained by another diagnosis. Recognizing these inflammatory signs and symptoms is crucial because EAM of RA is associated with increased mortality, so it is essential that a proper diagnosis be made and treatment initiated in a timely fashion to avoid as much permanent damage as possible. It is also important to be mindful of the possible need to collaborate with other specialists depending on which organ systems are affected so that the appropriate treatment regimen and follow-up plan can be put in place for the patient in order to achieve the best possible outcome. Early diagnosis and treatment with steroids to control acute RA flares are highly effective and other therapies, such as methotrexate, the first-line agent according to the American College of Rheumatology, can be initiated in conjunction or thereafter, typically with very good response, as demonstrated in the case presentation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Human Kallikrein-2 and Free Prostate Specific Antigen as Biomarkers for Early Detection of Prostate Cancer, Sudan: A Case-Control Study

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Abstract

Background: Prostate cancer (PCa) is considered one of the major health threats facing males in Sudan. Prostate-specific antigen (PSA) test is the most important laboratory test used in the diagnosis of prostate cancer, the main disadvantage of PSA is its limited specificity, which triggered a lot of interest in development, more research on other markers such as serum human kallikrein-2 (KLK-2) and free prostate specific antigen (fPSA). **Objectives:** To evaluate the validity of serum kallikrein-2 (KLK-2) and free prostate specific antigen (fPSA) in the early detection of prostate cancer among Sudanese patients. **Method:** In this study seventy men were considered as a case subject, who were diagnosed as cancer prostate at Gezira Hospital for Renal Disease and Surgery (GHRDS), Sudan during the period February 2018 to July 2019. Randomly selected sixty patients of BPH patients and forty-five apparently healthy men as control subject. KLK-2, fPSA and PSA estimations were performed from serum samples using the principle of Enzyme Linked Immunosorbent Assay (ELISA). **Results:** The results revealed a highly significant difference between the serum levels individual biomarkers (KLK-2, fPSA, PSA) and multiple biomarkers (fPSA/PSA, KLK-2/fPSA, KLK-2/PSA) for patients with prostate cancer when compared with the control groups. Furthermore, the fPSA/PSA ratio was lower in the patients with prostate cancer (P value = 0.00) than in the control group, the fPSA/PSA ratio showed that best accuracy to differentiate prostate cancer from control group, fPSA cut-off value was

found to be more than (18 ng/ml), with sensitivity (93%), specificity (80%), and odds ratio (55). **Conclusions:** The use of multiple biomarkers rather than individual biomarkers especially fPSA/PSA ratio improves the specificity as well as maintenance of higher sensitivity for early diagnosis of the prostate cancer.

Keywords

Kallikrein-2, PSA, fPSA, Prostate Cancer, Sudan

1. Introduction

The most important exocrine gland in male reproductive system is the prostate. The prostate gland is exposed to many disorders, the most important of which are tumors with their malignant and benign types [1]. The non-cancerous type is known as benign prostatic hyperplasia (BPH), the main change of this condition is the increase in size from the normal shape with subsequent symptoms associated with the obstructiveness [2].

Globally, prostate cancer ranked as the second cancer for men, and resulting in death for more than three hundred and fifty thousand cases; this is approximately equivalent to 3.8% of deaths from all types of cancer among men in 2018 [3].

There is a relationship between the incidence and mortality rate of prostate cancer globally, and age, with an average of 66 years at the time of diagnosis. For American men from African roots, there was a higher incidence of prostate cancer compared to white Americans. It should be noted that, more than one hundred and fifty-eight new cases were diagnosed for every one hundred thousand people, and the death rate among black Americans is two times than that of Americans white-skinned [4].

In southern Africa, prostate cancer was recorded per hundred thousand populations to be 66 to 111, as for North Africa and the middle countries such as Egypt, Algeria, Libya and Sudan; it gave a rate of less than 16.3 [5]. While the mortality rates recorded among 100.000 population reach 24.4, 18.7 and 7.0 in southern Africa, Eastern Africa and Northern Africa respectively [6] [7].

Based on the data issued by the Sudanese Radiation and Isotopes Center of Khartoum (RICK), prostate cancer is the most common male cancer [5], more than that the incidence to develop prostate cancer has increased in Sudan the past two decades [8]. These cases resulted in a mortality rate of 8.7 per hundred thousand people. It was also found in Sudan that, cases of prostate cancer (PCa) are related to age and sexual activity rather than race [6]; justifications for this disparity have been assumed to differences in social, environmental and genetic influences [9].

Prostate specific antigen (PSA) is a protein that synthesized in very large quantities only in the prostate gland. However, the concentrations of PSA avail-

able in the blood are so vary every day due to several influencing factors such as sexual intercourse and infections [10].

In 1994, PSA was approved as a tool for early detection of prostate cancer by the United States Food and Drug Administration (FDA) [11]. Similar to many serum tumor markers, PSA could be detected in both benign and malignant tumors. In cases of prostate cancer, a significant increase in PSA concentration in the blood was documented in localized and advanced conditions. There is a clear correlation between increased PSA concentrations and tumor size [12], however, it was also found that there is an overlap between PSA concentrations in the blood of both benign and malignant situations [11].

The PSA test is one of the most popular laboratory tests, and it provides results that facilitate the early detection of prostate malignant tumors, but this test has raised a lot of controversy in recent years [13]. Annual PSA screening with digital rectal examinations have been one of the most important recommendations proposed by the American Cancer Society and the American Urological Association (AUA) [14]. On the other hand, some organizations such as the National Cancer Institute, the American College of Physicians and the US Preventive Services Task Force recently issued some recommendations motivating not to use the PSA test because it offers few benefits in early detection of prostate cancer and the harm can be significant [15].

PSA test weakness for specificity may give false positive results, This, in turn, leads to an increase in the number of prostate biopsies taken for PSA cases whose results range from 4.0 to 10.0 ng/ml [16]. There are previous documented studies that showed that some cases that have normal PSA levels, but it is less than the level of 4.0 ng/ml, but it was found that they have prostate cancer, although there are many cases under this study that have PSA levels much higher than the normal range and they do not have a malignant tumor, this is due to only for the presence of a benign tumor in these cases [17]. According to these facts listed above, the urgent need to adopt studies that improve the specificity of PSA for early detection of prostate cancers has necessitated.

PSA is present in the circulatory system in different forms: complexed to alpha 1 anti-chymotrypsin (PSA-ACT complex), unbound (free PSA), and enveloped by alpha-2-macroglobulin [18]. In cases without an enlarged prostate, the vast majority of free (unbound) PSA is present in the serum reflects the mature protein that has been inactivated by internal proteolytic cleavage. In contrast, this cleaved fraction is relatively decreased in prostate cancer, this exposed why the concentrations of free PSA in the blood are much lower in men with prostate cancer [19].

On the contrary, it was found that the levels of complexed PSA concentrations among annually screened men were very high compared to their peers who had either normal prostate or benign tumor [20], this outcome has been exploited in the use of the ratio of free to total PSA and complexed PSA as means of distinguishing between PCa and BPH [21].

Human kallikrein 2 (KLK-2) is a kind of serine protease, which owns (79%) of the amino acid sequence identical with PSA. KLK-2 is also a novel biomarker for PCa early detection. Most of the produced quantities of KLK-2 are synthesized in the prostate gland, after which it is excreted outside in the form ofproenzyme in the body and outside the cell is activated into active enzymes. KLK-2 is present in varying concentrations in blood, semen, saliva and many other body fluids, in excess of (90%) KLK-2 is present in the circulation in a free, unbound form. Numerous experiments and researches have confirmed the hypothesis of KLK-2's ability to detect prostate cancer and prognosis [22].

Therefore, numerous studies designate that measurement of serum KLK-2 with PSA can improve diagnosis of prostate cancer, importantly, the ratio of KLK-2/fPSA ratio alone or combined with fPSA/PSA ratio enhance the differentiation between prostate cancer and BPH [23].

While those biomarkers still have disadvantage or show some weakness, until now, no individual biomarker has been demonstrated to be more valuable in PCa than PSA, in this study. Early diagnosis of prostate cancer in Sudan faces many obstaclessuch as the limitation of diagnoses logistics, inadequacy of health, life-style changes and economic instabilities in Sudan [24]. Therefore, we investigated the role of serum KLK-2, free PSA to differentiate prostate cancer from benign diseases in Sudanese patients.

2. Patients and Methods

A case control study was conducted which included prostate cancer patients, benign prostate hyperplasia patients and apparently healthy individuals. Serum-Kallikrein-2 (KLK-2), free Prostate Specific Antigen (fPSA) and Prostate Specific Antigen (PSA) biomarkers were determined in serum samples from case and control subjects. In this study, seventy men were considered as a case subjects, who were diagnosed as cancer prostate at Gezira Hospital for Renal Disease and Surgery (GHRDS), Sudan during the period February 2018 to July 2019.

Sixty patients of BPH patients and forty-five apparently healthy men were randomly selected as a control group. The diagnosis of PCa and BPH was based on the clinical evaluation, biochemical (PSA, fPSA, and KLK-2) and prostate tissue biopsy.

The inclusion criteria were men 40 years old and more, the cases subjects were newly diagnosed and they did not receive any kind of treatment during samples collection; while patients with prostatitis, sexually transmitted infections, patients with chronic renal failure, patients on finasteride or dutasteride therapy for the prostatic disease were excluded. The control included BPH and healthy patients were included only in this study.

We used an interviewer administered questionnaire to ask the cases and the controls about their demographic, socioeconomic, and geographical afflation, as well as clinical data including family history of prostate cancer. Laboratory investigations data were also recorded.

3. Sample Processing and Analysis

Five ml of blood samples were collected into plain container from the subjects in a relaxed mood without any prior prostate manipulation. The blood was centrifuged within 20 minutes after collection at 3000 revelations per minute for 10 min, and serum used for PSA, KLK-2 and fPSA analyses. The samples were stored at −70˚C until analysis. KLK-2, fPSA and PSA was measured using the sandwich Enzyme Linked Immunosorbent Assay (ELISA) by full-automated machine (ELITE) and fortress kits (LOT: FPS-1902-1) according to the manufacturer's recommendations.

For statistical analyses the Statistical Software Package (version 24) for Windows (SPSS, Chicago, IL, USA) was used. The non-parametric Kruskal Wallis test of variance was carried out. A two-sided P value lower than 0.05 was considered statistically significant. Correlation analyses between variables were determined using Pearson's bivariate correlation test, also formula for sensitivity, specificity, odd ratio, positive and negative predictive values were achieved.

The reference ranges for PSA are considered normal up to 4.0 ng/ml. Since there were no reference values for fPSA and KLK-2 because they were used for research purposes, a high reading was taken compared to the control group.

All protocols involving human subjects were reviewed and approved by the ethical committee of University of Gezira. Informed written consents from the human subjects were obtained in this study.

4. Results

In total, 180 participants were enrolled; 75 were prostate cancer patients, 60 were BPH patients and 45 were apparently healthy subjects. The means of age of PCa group, BPH and apparently healthy subjects were 66.96 years, 72.46 years, and 74.93 years respectively. In prostate cancer group, 74.7% (56/75) of patients were located in rural areas while observable risk factors was smoking 18.7% (14/75), positive family history 18.7% (14/75), 30.6% (23/75) as farmers and 33.3% (25/75) as workers.

The range of PSA values was from 4.62 to 1030 ng/ml in PCa patients, with a mean ± SE of 67.52 ± 123.66 ng/ml and from 2.45 to 92.78 ng/ml in BPH patients, with the mean ± SE value being 15.41 ± 20.68 ng/ml. In apparently healthy group the mean ± SE of 1.93 ± 1.04 ng/ml with the concentration's values fell between 0.33 to 3.80 ng/ml. The fPSA values ranged from 0.48 to 122 ng/ml in PCa patients, with a mean ± SE of 8.04 ± 15.04 and from 0.44 to 35.12 ng/ml in BPH patients, with the mean ± SE value being 4.15 ± 6.77. The fPSA in apparently healthy group range fell between 0.1 to 1.22 ng/ml with the mean ± SE value being 0.48 ± 0.29. The KLK-2 values werefrom 0.03 to 2.33 ng/ml in PCa patients, with a mean ± SE of 0.41 ± 0.54 ng/ml and from 0.02 to 0.24 ng/ml in BPH patients, with the mean ± SE value being 0.22 ± 0.24 ng/ml, and in apparently healthy group had mean ± SE of 0.15 ± 0.13 ng/ml with the concentration values from 0.02 to 0.51 ng/ml. The fPSA/PSA ratio ranged from 5.91 to

67.13 (%) in PCa patients, with a mean ± SE of 12.57 ± 7.31 (%) and from 8.39 to 45.36 (%) in BPH patients, with the mean ± SE value being 23.66 ± 7.50 (%), The fPSA/PSA ratio in apparently healthy group range fell between 13.61 to 43.08 (%) with the mean ± SE value being 25.83 ± 7.75 (%). The range of KLK-2/PSA ratio from 0.05 to 1.94 (%) in PCa patients, with a mean ± SE of 0.661 ± 0.49 (%) and from 0.39 to 4.62 (%) in BPH patients, with the mean ± SE value being 1.52 ± 1.01 (%), and in apparently healthy group had mean ± SE of 7.61 ± 4.13 (%) with the concentration values from 2.91 to 22.12 (%). The KLK-2/fPSA ratio ranged from 0.272 to 19.95 (%) in PCa patients, with a mean ± SE of 6.03 ± 4.17 (%) and from 1.50 to 39.57 (%) in BPH patients, with the mean ± SE value being 7.47 ± 6.70 (%), The KLK-2/fPSA ratio in apparently healthy group range fell between 8.15 to 58.38 (%) with the mean ± SE value being 30.33 ± 13.81 (%) (Table 1).

To compare the validity measurement among all the biomarkers and specify the mostsuitable cut-off value for each biomarker, sensitivity, specificity, odd ratio, positive and negative predictive value were calculated and illustrated. This applied to locate the most viable biomarkers as a panel of performed biomarkers tool for participating in the detection of prostate cancer. The best sensitivity (78%) recorded with fPSA while best specificity equal 76.3% and showed with PSA. All examined biomarkers significantly differentiated between the cancer and control groups (Table 2).

Table 1. Statistical analysis of serum PSA, fPSA, KLK-2 and their ratios in the three different subjects. No 180.

Group Factors	Prostate cancer (N = 75) Mean ± SD (range)	Prostatic hyperplasia (N = 60) Mean ± SD (range)	Apparently healthy (N = 45) Mean ± SD (range)
PSA ng/ml	67.52 ± 123.66 (4.62 - 1030)	15.41 ± 20.68 (2.45 - 92.78)	1.93 ± 1.04 (0.33 - 3.80)
fPSA ng/ml	8.04 ± 15.04 (0.48 - 122)	4.15 ± 6.77 (0.44 - 35.12)	0.48 ± 0.29 (0.10 - 1.22)
KLK-2 ng/ml	0.41 ± 0.54 (0.03 - 2.33)	0.22 ± 0.24 (0.02 - 0.74)	0.15 ± 0.13 (0.02 - 0.51)
fPSA/PSA (%)	12.57 ± 7.31 (5.91 - 67.13)	23.66 ± 7.50 (8.39 - 45.36)	25.83 ± 7.75 (13.61 - 43.08)
KLK-2/PSA (%)	0.661 ± 0.49 (0.05 - 1.94)	1.52 ± 1.01 (0.39 - 4.62)	7.61 ± 4.13 (2.91 - 22.12)
KLK-2/fPSA (%)	6.03 ± 4.17 (0.272 - 19.95)	7.47 ± 6.70 (1.50 - 39.57)	30.33 ± 13.8 (8.15 - 58.38)

PSA: Prostate Specific Antigen, **fPSA**: Free Prostate Specific Antigen, **KLK-2**: Kallikrein-2, **N**: number of cases, **SD**: Standard deviation.

As shown in Table 3, regarding PCa group versus control group, fPSA/PSA is the best one with sensitivity, specificity, PPV, NPV, OR of 93%, 80%, 76%, 94% and 55 respectively. All ratios were highly significant for differentiation of prostate cancer group compared to control groups with P. value of 0.00.

Table 2. The cut off, sensitivity, specificity, PPV & NPV and odds ratio for KLK-2 and fPSA in malignant prostate tumor and control groups.

Parameter	PSA	KLK-2	fPSA
Cut off (ng/ml)	>7.84	>0.085	>1.44
Sensitivity%	69%	58%	78%
Specificity%	76.3%	55%	70%
PPV%	71.3%	48%	65%
NPV%	75.2%	65%	82%
O.R	11.01	1.71	8.65
P. value*	0.04	0.02	0.02

PSA: Prostate specific antigen, **KLK-2**: Kallikrein-2, **fPSA**: free Prostate Specific Antigen, **PPV**%: Positive Predictive Value%, **NPV**%: Negative Predictive Value%, **O.R**: Odds Ratio, **P. value**: P. value ≤ 0.05 is considered as significant. *By Kruskal Wallis Test (Non parametric test).

Table 3. The cut off, sensitivity, specificity, PPV & NPV and odds ratio for fPSA/PSA, KLK-2/PSA and KLK-2/fPSA in malignant prostate tumor and control groups.

Parameter	fPSA/PSA (%)	KLK-2/PSA (%)	KLK-2/fPSA (%)
Cut off (ng/ml)	>18	>1.032	>7.218
Sensitivity%	93%	83%	67%
Specificity%	80%	73%	62%
PPV%	76%	69%	55%
NPV%	94%	86%	73%
O.R	55	14.2	3.38
P. value*	0.00	0.00	0.00

fPSA/PSA (%): Free toProstate Specific Antigen (%); **KLK-2/PSA (%)**: Kallikrein-2 to Prostate Specific Antigen (%); **KLK-2/fPSA (%)**: Kallikrein-2 to Free Prostate Specific Antigen (%); **PPV**%: Positive Predictive Value%; **NPV**%: Negative Predictive Value%; **O.R**: Odds Ratio: **P. value**: P. value ≤ 0.05 is considered as significant. *By Kruskal Wallis Test (Non parametric test).

5. Discussion

Prostate disease is a specified only to men and usually affects advanced ages, and the most common types are benign prostatic hyperplasia, prostatitis and prostate cancer. Many western studies regarding the biomarker diagnostic indicators for prostate diseases have documented that, there is no specific test that can be inferred to notify the patient that he has prostate cancer, but the specificity could be improve by combining results from more than one biomarker. The benefits of this combination are statistically stronger outcomes and reduced number of unnecessary biopsies [25].

This study revealed that prostate cancer is principally a disease of older men and is infrequent below the age of 45 years. This goes with the statements by Cao *et al.*, they stated that prostate cancer incidence increases intensely with advanced ages. Although it is a very unusual disease in men younger than 45 years, rates increase exponentially thereafter [26].

In this study, the mean prostate specific antigen level has a significant increase in the prostate cancer group compared with the control subjects (67.52 ± 123.66 ng/ml) ($P = 0.04$) and this reflects the ability of PSA in distinguishing persons with prostate cancer from other persons without PCa. This come to an agreement with a study done by Sajjad *et al.* [18], who found a significant variance in the mean of serum level of prostate specific antigen between the patient's group (25.7 ± 21.6) and control group (12.7 ± 6.9) ng/ml ($P = 0.01$), the reason due to the odd outflow of PSA into the blood circulation which is occur by the level of PSA expression in malignant epithelium and by distortion in structure of prostatic glandular [27].

Only in advance stages of malignant prostate cases show higher levels results of PSA, Therefore, the PSA surpass the gray zone between (4 - 10 ng/ml). Thus, the specificity of PSA as a tumor marker is limited. The determination of the proportion of fPSA has been widely used to expand the specificity of PSA, especially in gray zone, in which the serum PSA values for BPH and PCa commonly overlap [28].

There are no documented scientific data on the uses of KLK-2 and fPSA serum assays in patients with benign or malignant disorder in Sudan, so this study is considered as the first in Sudan in this type of study and results, so the results of this study were compared with the results from other non-Sudanese in previous studies.

Free prostate specific antigen is usually reduced in prostate cancer than in benign prostatic hyperplasia, a ratio of fPSA to PSA (fPSA/PSA) greater than 25% lowers the chance of prostate cancer compared with a % of PSA ≤ 10% [29], according to correlation results which showed same corresponding correlation between PSA levels and fPSA levels, also indicated reverse correlation between PSA levels and fPSA/PSA ratio, due to the abnormal leakage of fPSA into the circulation influenced by the level of fPSA expression in malignant epithelium, the results in our study was agreed with many studies [25].

On the other hand, the results gave noteworthy observation that most of the BPH and apparently healthy group had highly fPSA/PSA ratio 23.66 ± 7.50(%) and 25.83 ± 7.75 (%) respectively, but in contract the PCa cases had fPSA/PSA ratio 12.57 ± 7.31 (%). These results were confirmed by the several distinctive studies which mentioned that 70% of the subjects diagnosed for PCa were to be positive results using fPSA/PSA, so fPSA/PSA ratio significantly improved the capability to differentiate between PCa and patients having BPH or apparently healthy subjects as compared to PSA only, Therefore, fPSA/PSA ratio is useful predictor for early detection of PCa [16] [18] [30].

Our findings are similar and corresponding to the past studies which testified that some Kallikreins family are raised in many malignant disorder, especially KLK-2 which increase significantly in prostate cancer so it may be used as a marker in this type of cancer [31] [32]. Subsequently, previous studies validate the utility of the ratio KLK-2/fPSA, and KLK-2/PSA to discriminate prostate cancer patients from non-cancer men, expressing that this ratio may have a useful early diagnostic role in malignant prostate [28].

According to study results that showed elevated levels of PSA, fPSA and KLK-2 with p = (0.04, 0.02, 0.02) respectively among the study groups which can be used as differentiating tool between prostate cancer and controls. Furthermore, significant elevated levels of fPSA/PSA with p = (0.00) among prostate cancer group, that give it qualities as powerful tool for detection the prostate cancer condition.

On the contrary, KLK-2/PSA and KLK-2/fPSA are significantly elevated among control groups with p = (0.00, 0.00) respectively, that give it qualities as full power tool for detection non-malignance conditions, and also give a strong indication for the benefit of multiple biomarkers for detection and differentiation between prostate cancer and control groups.

The findings of this current study showed that the PSA cut-off value was (>7.84 ng/ml) for prostate cancer patients. According to the American Cancer Society, PSA level above 4 ng/mL and below 10 ng/mL have 25% chance of PCa occurrence and PSA level more than 10 ng/mL increases chances of PCA occurrence over 50% [30]. Although, the PSA test play an important role in the early detection of prostate cancer cases, but it is still difficult to describe it as the ideal biomarker, due to the fact that there is no accurate threshold that can be used with confidence for the diagnosis of PCa.

Study results, showed the using KLK-2/PSA and KLK-2/fPSA ratios enhance sensitivity and specificity of prostate cancer detection than using KLK-2, fPSA and PSA alone, which our results in the line with Kwiatkowski *et al.* who found that the ratio of KLK-2/fPSA, not KLK-2 or fPSA alone, improved cancer detection [33].

In addition, fPSA/PSA ratio showed highest sensitive value 93% and specific value 80% compared with PSA alone which has sensitivity value 69% and specificity value 76.3%, even fPSA/PSA ratio showed high sensitivity and specificity more than other multiple biomarkers KLK-2/fPSA, and KLK-2/PSA. fPSA/PSA

ratio was lessened in the patients with malignant prostate compared in the patients with benign and apparently healthy prostate conditions,

Various studies were showed that fPSA/PSA ratio was lessened in patients with PCa than in patients with benign or other prostatic diseases. Concurrent immune detection of both PSA, fPSA and fPSA/PSA ratio helps in differentiation of PCa from other uncancerous conditions of prostate. Thus it increases the specificity and sensitivity as well as diagnostic accuracy in detecting prostate cancer [34].

It has also been demonstrated that reduced fPSA/PSA ratio are seen in the cases of PCa with histopathologically higher Gleason Score. Thus an inverse correlation between the fPSA/PSA ratio and histopathological aggressiveness of PCa has established which also contributes to the higher specificity of the serum test [35].

6. Conclusion and Recommendation

Differentiation of men with and without PCa in a randomly selected population was improved by measuring individual biomarkers such as KLK-2, PSA and fPSA, furthermore we have found that use of multiple biomarkers such as fPSA/PSA, KLK-2/fPSA and KLK-2/PSA gave higher diagnostic accuracy better than use of one biomarker alone, with the fPSA/PSA as the best multiple biomarker. Above that, KLK-2 and fPSA measurements are simple, non-invasive, and relatively inexpensive procedures, and could be used as a routine test for prostate cancer diagnoses.

Study Limitation

Obstacles and shortcomings that encountered during the conducting of this research, which must be mentioned; were the relatively small sample size due to scarcity of cases in hospitals, the difficulty of finding financial sources from other parties to fund our private research and the short-term storage of tested serum samples for KLK-2 and fPSA measurement.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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