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Measurement of D-dimer, Platelets Count and Indices and Platelet Lymphocyte Ratio among Sudanese patients of Prostate Cancer in Khartoum State

قياس مستوى دي دايمر, عدد الصفائح الدموية و مؤشراتها و نسبة الصفائح الدموية الى الخلايا الليمفاوية بين مرضى سرطان البروستاتا السودانيين في ولاية الخرطوم

A thesis submitted for partial fulfillment of the requirement of M.Sc. degree in Medical Laboratory Science (Hematology and Immunohematology)

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الآية

بسم الله الرحمن الرحيم

﴿ وَقَالُوا الْحَمْدُ لِلَّهِ الَّذِي أَذْهَبَ عَنَّا الْحَزَنَ ۖ إِنَّ رَبَّنَا لَغَفُورٌ شَكُورٌ ﴾

صدق الله العظيم
سورة فاطر – الآية [34]

Dedication

THIS THESIS IS DEDICATED TO MY PARENTS, MY SISTERS AND MY BROTHERS FOR THEIR ENDLESS LOVE, SUPPORT AND ENCOURAGEMENT.

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In the name of Allah, the most gracious and most merciful. First and foremost I am thankful to **Almighty Allah** for giving me the strength, knowledge, opportunity and ability to undertake this study and complete it satisfactory.

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Abstract

This was a case control study conducted at Khartoum hospital during the period from April to October 2022, aimed to measure D-dimer, platelets count and indices, and platelet to lymphocyte ratio among Sudanese patients of prostate cancer in Khartoum state. A total of 100 subjects were involved in this study, 50 prostate cancer patients as cases and 50 healthy volunteers as control (1:1) matched age and sex. Data were collected using a pre-structured questionnaire filled by direct interview with participants. Venous blood (4.3 ml) was collected from each participant and distributed in two containers, 2.5 ml EDTA sample was analyzed by Sysmex automated hematology analyzer for measurement of complete blood count and 1.8 ml in trisodium citrate containers for measuring D- dimer level. Data were analyzed by statistical of package social science (SPSS), version 25. The statistical analysis was performed by using independent t-test and chi-square test (A P-value of < 0.05 was considered significant). Result showed that D-dimer level was significantly increased in patients with prostate cancer compared with control group (1.72µg/ml) and (0.18±0.07) with P-value (0.00), MPV, PDW, PLCR and PCT were significantly higher in prostate cancer patients compared with control means (10.20±0.88 fl) vs. (8.06±0.81 fl), (11.81±2.06 fl) vs. (10.49±1.04fl), (26.61±7.15%) vs. (14.64±5.17%) and (0.26±0.10%) vs. (0.19±0.04%) respectively, (P value = 0.00). There was no significant difference between two groups in regard to platelets count, PLR (p-value >0.05). The study also showed that patients who received chemotherapy had higher PDW (p-value 0.05).

In conclusion, patients with prostate cancer had higher plasma D-dimer level, MPV, PDW, PCT and PLCR. PDW were higher in prostate cancer patients receiving chemotherapy.

المستخلص

هذه دراسة حالة شواهد أجريت في مستشفى الخرطوم خلال الفترة من أبريل إلى أكتوبر 2022 ، تهدف إلى قياس ، عدد الصفائح الدموية ومؤشراتها ، ونسبة الصفائح الدموية إلى الخلايا الليمفاوية بين مرضى سرطان البروستاتا السودانيين في ولاية الخرطوم. تم إشراك ما مجموعه 100 شخص في هذه الدراسة ، 50 مريضًا بسرطان البروستاتا كحالات و 50 متطوعًا سليمًا كمجموعة تحكم (1: 1) متطابقة في العمر والجنس. تم جمع البيانات باستخدام استبيان منظم مسبقًا مملوء بمقابلة مباشرة مع المشاركين. تم جمع الدم الوريدي (4.3 مل) من كل مشارك ووزع في عبوتين ، تم تحليل 2.5 مل من EDTA بواسطة محلل الدم لقياس تعداد الدم الكامل و 1.8 مل في حاويات سترات الصوديوم لقياس مستوى دي- دايمر. تم تحليل البيانات بواسطة إحصائية حزمة العلوم الاجتماعية (SPSS) ، الإصدار 25. تم إجراء التحليل الإحصائي باستخدام اختبار t المستقل واختبار مربع كاي (اعتبرت قيمة الاحتمال أقل من 0.05ذات اهمية احصائية). أظهرت النتائج أن مستوى دي دايمر زاد بشكل ملحوظ في مرضى سرطان البروستاتا مقارنة مع مجموعة التحكم (1.72 ميكروغرام / مل) و (0.18 ± 0.07) بقيمة (0.00) P، مؤشرات الصفائح الدموية كانت أعلى بشكل ملحوظ في المرضى الذين يعانون من سرطان البروستاتا مقارنة مع مجموعة التحكم، (قيمة الاحتمال = 0.00). لم يكن هناك فرق كبير بين مجموعتين فيما يتعلق بعدد الصفائح الدموية و نسبة الصفائح الدموية الى الخلايا الليمفاوية (قيمة 0.05 <p). أظهرت الدراسة أيضًا أن المرضى الذين تلقوا علاجًا كيميائيًا كان لديهم ارتفاع في عرض توزيع الصفائح الدموية (قيمة 0.05 p).

اشارت المحصلة ان المرضى المصابين بسرطان البروستات لديهم مستوى أعلى في البلازما دي دايمر و مؤشرات الصفائح الدموية. و ايضا عرض توزيع الصفائح الدموية كان أعلى في مرضى سرطان البروستاتا الذين يتلقون العلاج الكيميائي.

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List of Abbreviations

Abbreviations	Terms
ABP	Acute bacterial Prostatitis
ADP	Adenosine di phosphate
ADT	Androgen deprivation therapy
AR	Androgen receptor
ATP	Adenosine tri phosphate
BHP	Benign hypertrophy of prostate
BHP	Benign prostatic hyperplasia
CBP	Chronic bacterial Prostatitis
CRP	C-reactive protein
CRPC	Chronic pelvic pain syndrome
CRPC	Castration resistant prostate cancer
DER	Digital rectal examination
DIC	Disseminated intravascular coagulation
FDP	Fibrin degradation product
GP	Glycoprotein
HSC	Hemopoetic stem cell
HSPC	Hormone sensitive prostate cancer
HSPC	Hormone sensitive prostate cancer
IFN	Interferon
LMR	Lymphocyte monocyte ratio
MKS	megakaryocytes
MPV	Mean platelet volume
MRI	Magnetic resonance imaging
MSR- 1	Macrophage scavenger receptor -1
NLR	Neutrophil lymphocyte ratio
OS	Overall survival

PCa	Prostate cancer
PCT	Plateletcrit
PDW	Platelet distribution width
PI	Platelet indices
PLCC	Platelet large cell count
PLCR	Platelet large cell ratio
PLR	Platelet lymphocyte ratio
PLT	Platelet
PSA	Prostate specific antigen
PTIS	Prostatitis
SPSS	statistical of package social science
TCIPA	Tumour induced platelet aggregation
TRUS	Trans rectal ultrasound
TXA	Thromboxane
VTE	Venous thromboembolism
VWF	Von Willebrand factor

CHAPTER I

Introduction

Chapter I

1. Introduction

1.1 Introduction

Cancer is considered one of the leading causes of morbidity and mortality, throughout the world, with approximately 19.3 million new cases in 2020; this number is expected to be 28.4 million cases in 2040 which represent about 47% rise from 2020; with approximately 10.0 million cancer deaths occurred in 2020 that make cancer the second most common cause of death globally (Sung *et al*., 2021).

Prostate cancer is the second most common cancer among men worldwide and has become a significant health problem in both developed and developing countries (Caliskan and Sungur, 2017).

Prostate cancer is the fourth leading cause of death in men worldwide, although prostate cancer can be managed in the early stages by surgical intervention or radiation, with time; approximately 7.0% of cases of prostate cancer metastasize and progress to castration-resistant cancer, even with aggressive hormone deprivation therapy. The prognosis is poor in men with distant metastases of prostate cancer, in which bone is the most common site of involvement (Sağlam and Çimen, 2021).

Recent investigations showed that platelets play critical roles in mediating the growth, dissemination, and angiogenesis of cancer cells (Sharma *et al*., 2014). Activated platelets are associated with cancer progression and metastasis (Goubran *et al*., 2014). Mean platelet volume (MPV) and platelet distribution width (PDW) are markers of activated platelets and are associated with gastric cancer, ovarian cancer, lung cancer, colon cancer, and breast cancer (Yin *et al*., 2020). MPV and PDW have been shown to have diagnostic values for these cancers. Several studies also revealed the association of MPV and PDW with prostate cancer, reported that decreased MPV and increased PDW were found in patients with prostate cancer, concluded that MPV and PDW combined with PSA could differentiate PCa patients (Fukuokaya *et al*., 2020).

Cancer patients may have increased risk of thrombosis and may be associated with high levels of coagulation markers such as fibrinogen and D- dimer as thrombogenesis

markers, although the activation of coagulation cascade contributes to tumor progression and metastasis, cancers induce a hypercoagulable state that promotes venous thromboembolism (VTE), which is a frequent complication and a leading cause of morbidity and death in patients with cancer (Posch *et al*., 2020), D-dimer is a degradation product of fibrin which is produced by plasmin-induced fibrinolysis, high level of D-dimer is a prognostic factor associated with increased mortality risk in patients with brain tumors, lymphomas, and breast, lung, stomach, colorectal, pancreatic, and prostate cancers (Çalışkan and Sungur, 2017).

Inflammation and immunity response play a critical role in most chronic diseases, Platelet to lymphocyte ratio (PLR) is a novel inflammatory marker that can be applied in many diseases for predicting inflammation, it is found to be a useful indicator of inflammation in a wide spectrum of diseases other than sepsis like acute myocardial infarction, acute kidney injury, cancer condition like hepatocellular carcinoma and nonsmall cell lung cancer (Kutlucan *et al*., 2016). Platelet lymphocyte ratio simply calculated from the absolute platelet count and the absolute lymphocyte count, role of PLR as a prognostic marker was first reported in neoplastic diseases like hepatocellular carcinoma and breast cancer and associated with increased hospital mortality (Shen *et al*., 2019). Zheng et al reported that both high and low PLRs are associated with increased mortality in critically ill patients (Zheng *et al*., 2017). Application PLR for predicting clinical prognosis has been universally applied to patients with cancer, coronary artery disease, and subarachnoid hemorrhage (Graziano *et al*., 2019). Several studies reported that inflammation and immune system are related to prostate cancer and proposed platelet-to-lymphocyte ratio (PLR) as novel indices help in diagnose prostate cancer (Xu *et al*., 2021), also high platelet to lymphocyte ration (PLR) identified to be associated with poor prognosis in prostate cancer (Zhang *et al*., 2019).

The aim of this study is to evaluate D-dimer, platelets count and indices and platelet lymphocyte ratio for their prognostic values in Sudanese patients with prostate cancer.

1.2 Rationale

Cancer is a leading cause of death around the world, responsible for nearly 10 million deaths in 2020 (Sung *et al*., 2021). Globally, prostate cancer is the second most commonly diagnosed male malignancy and the 5th leading cause of cancer death in men (Rawla, 2019). In Sudan, the record showed that breast cancer is the most common tumor in females, while in males the prostate cancer is on top of the incidence (Maki, 2022).

Cancer and increased age are among the major risk factors for coagulation activation and thrombosis. Disorders of hemostasis are commonplace in patients with prostate cancer and include disseminated intravascular coagulation, venous thromboembolism and postsurgical bleeding. D.dimer is a degradation product of fibrin and serves as a valuable marker of activation of coagulation and hemostasis. To the best of our knowledge there is no published data addressing the association between D-dimer, platelets count and indices and platelet to lymphocyte ratio (PLR) in Sudanese patients with prostate cancer. So this study aimed to fill the gap and add new knowledge for using these parameters as prognostic markers for prostate cancer.

1.3 Objectives

1.3.1 General objective

To measure D-dimer level, platelets count and indices and platelet to lymphocyte ratio (PLR) among Sudanese patients with prostate cancer.

1.3.2 Specific objectives

1. To estimate D.dimer, platelets count and indices in patients with prostate cancer as cases and healthy subjects as control.
2. To calculate platelet lymphocyte ratio (PLR) in patients with prostate cancer and control group.
3. Association the hematological results with management protocol and metastasis.

CHAPTER II

Literature Review

Chapter II

2. Literature Review

2.1 Prostate

Prostate gland is the male sexual gland which is present in front of the rectum and between the bladder and penis; the main function of adult prostate is to secrete an opalescent aqueous liquid representing 10–30% of the seminal fluid volume in addition to citrate, spermine, proteases, immunoglobulins, prostaglandins and zinc, also the prostatic secretion contains a high concentration of acid phosphatase secreted by prostatic epithelial cells (Daniyal *et al*., 2014), (Giacomini *et al*., 2021).

2.1.1 Prostate Location

The prostate lies between the bladder and urogenital diaphragm, behind the lower of pubic symphysis and in front of ampulla of the rectal (Kulkarni, 2011), it is located in the pelvis, sits below the urinary bladder and surrounds the urethra. The part of the urethra passing through it is called the prostatic urethra, which joins with the two ejaculatory ducts (Young, *et al*., 2016).

2.1.2 Prostate Structure

The prostate composed of stroma and glandular tissue; the stroma composed of fibrous tissue and smooth muscle, the prostate stroma smooth muscle is continuous above with the smooth muscle of bladder wall. A peculiar feature of this muscle is the presence of striated muscle subjacent to the true capsule; striated muscle band is in continuity with the striated muscle of sphincter urethrae muscle surrounding the membranous urethra in the deep perineal pouch. The intraprostatic striated muscle is connected to the true capsule and stroma by collagen tissue. The intraglandular smooth muscle function is to compress the follicles to facilitate their drainage in prostatic urethra and the function of the striated muscle is probably to expand the prostatic urethra to accommodate seminal fluid (3–5 ml) prior to ejaculation (Ittmann, 2018). The second structural prostate component is the glandular tissue which arranged in three zones; the peripheral zone, the internal zone and the innermost zone. The peripheral zone consists of long branching glands with ducts curve to reach the posterior wall of the prostatic sinuses below the level of colliculus seminalis. The internal zone consists of sub mucosal glands, whose ducts open on the floor of prostatic sinuses at the level of colliculus seminalis. The sub mucosal

glands are prone to benign hypertrophy of prostate (BHP). The innermost zone consists of simple mucosal glands surrounding the upper part of the prostatic urethra (Kulkarni, 2011, Aaron, *et al*., 2016).

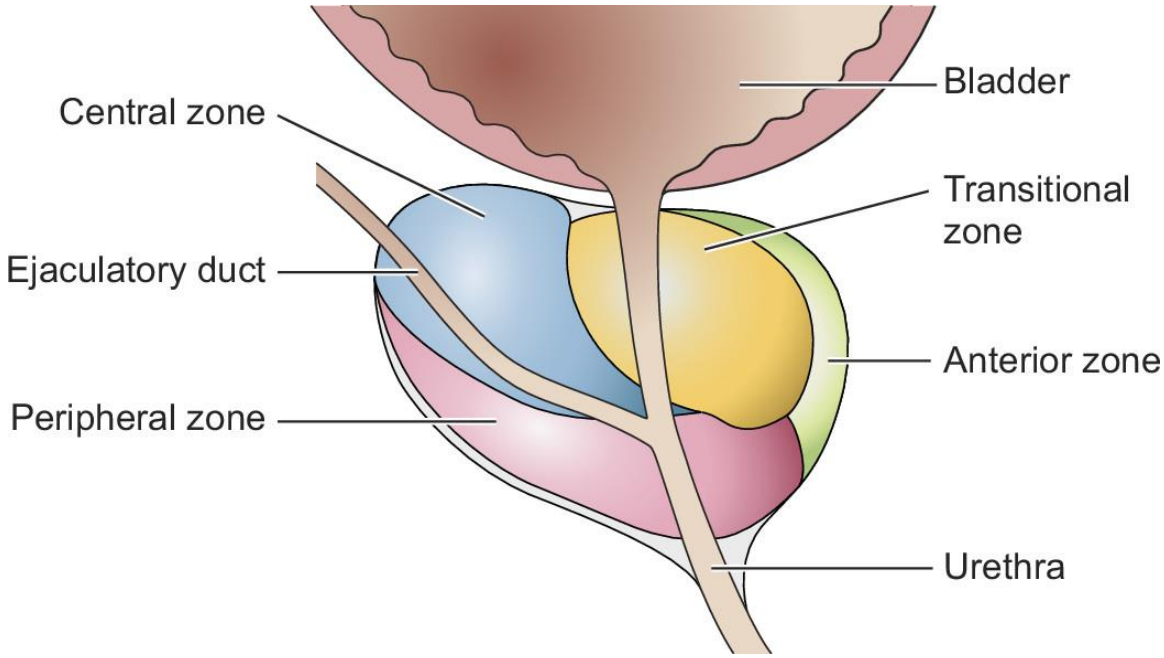

Figure 2.1 Anatomy of human prostate (Giacomini, *et al*., 2021)

2.1.3 Histological structure

The human prostate glandular epithelium is composed of acini and ducts lined by three types of cells; luminal, basal, and neuroendocrine. The acini have an undulating to papillary appearance in most cases; papillary configuration is noticeably more pronounced in the central zone. The luminal cells are columnar with pale eosinophilic cytoplasm and round nuclei near the base of the cell and luminal cells are specialized cells that secrete a variety of products into the lumen that contribute to the formation of the seminal fluid, these products include prostate-specific antigen (PSA), the luminal cells are strongly positive for PSA Immunohistochemistry. The basal cells are adjacent to

the basement membrane and have ovoid nuclei and inconspicuous cytoplasm, number of basal cells can be variable between glands in an individual prostate (Ittmann, *et al*., 2018). The prostatic stroma is fibro muscular, with abundant smooth muscle cells admixed with fibroblasts, blood vessels, and nerves, no adipose tissue is present in the prostate, skeletal muscle fibers are predominantly outside the prostate but often extend into the outer portion of the prostate as well (Ittmann, *et al*., 2018).

2.1.4 Prostate Physiology

The main role of the prostate as a male reproductive organ is to produce prostatic fluid that accounts for up to 30 percent of the semen volume, prostatic fluid promotes sperm motility and it is a milky color, alkaline fluid containing PSA, citric acid, calcium, zinc, acid phosphatase and fibrinolysin among its many constituents. During ejaculation, alpha-adrenergic stimulation of prostatic smooth muscle expresses seminal fluid containing sperm from the ampulla of the vas deferens into the posterior urethra, abnormal growth of the prostate is only experienced by humans and dogs, and why other mammals are spared is a mystery (Lawrentschuk *et al*., 2021).

2. 1.5 Prostate Diseases

Prostate abnormalities include; Prostatitis (PTIS), benign prostatic hyperplasia (BPH), prostate cancer (PCa) (Lokant and Naz, 2015).

2.1.5.1 Prostatitis

Prostatitis is still an enigmatic disease due to the inconsistency of the epidemiological data, the uncertainty etiology and the inadequacy of the diagnosis and the absence of a standardized pharmacological treatment. Prostatitis term literally refer to inflammation of the prostate, currently is used to describe a set of clinical conditions of uncertain etiology that are not always associated with a clear demonstration of the presence of an inflammatory process and that include painful pelvic symptoms, low urinary tract symptoms and sexual disorders that require differentiated treatment (Magri *et al*., 2018).

2.1.5.1.1 Acute bacterial Prostatitis (ABP)

ABP represents an acute infection of the prostate gland, generally acute bacterial Prostatitis is rare but when it occurs, it is often associated with bladder outlet obstruction or an immunocompromised condition (Davis and Silberman, 2021). ABP is associated with severe, mainly Gram-negative infection; it is characterized by an acute pain onset

combined with irritative and obstructive voiding symptoms in a patient with manifestations of a systemic febrile illness, Gram-negative bacteria can be easily isolated from the urine, *E. coli* is the most common pathogen encountered in ABP, accounting for 50%–87% of cases (Matsumoto and Yamamoto, 2021).

2.1.5.1.2 Chronic Prostatitis

Chronic Prostatitis is divided into different categories: a combination of chronic bacterial Prostatitis (CBP), chronic pelvic pain syndrome (CPPS), or asymptomatic Prostatitis (Pirola et al., 2019). Chronic Prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a complex of symptoms including urological pain, with or without voiding symptoms. The symptoms resemble those of a prostate infection, but an infectious etiology is found in only 5–10% of cases (Pontari, 2020).

2.1.5.2 Benign Prostatic Hyperplasia

Benign prostatic hyperplasia (BPH) is defined by the American Urological Association as a histologic diagnosis referring to the proliferation of smooth muscle and epithelial cells within the prostatic transition zone. The prostatic transition zone makes up about 5% of the prostate and is the portion that surrounds the proximal urethra. This zone is the site of continual growth throughout life (Lokeshwar *et al*., 2019).

2.1.5.3 Prostate Cancer

Prostate cancer represents the second most common cancer in men worldwide and the fifth most common cause of cancer death in men; in the United States (Sung, *et al*., 2021, American Cancer Society 2022), (Shah and Zhou, 2019). World Health Organization in 2016 classification provides a comprehensive listing of prostate tumors, including acinar adenocarcinoma subtypes (Humphrey, *et al*., 2016).

2.1.5.3.1 Epidemiology of Prostate Cancer

Prostate cancer is a major cause of morbidity and mortality among men and each year 1.6 million men are diagnosed with and 366,000 men die of prostate cancer (Pernar et al., 2018), prostate incidence increases with age, it constitutes 15% of carcinomas seen in male population (Kasap et al., 2021), only 1 in 350 men under the age of 50 years will be diagnosed with prostate cancer, the incidence rate increases up to 1 in every 52 men for ages 50 to 59 years. The incidence rate is nearly 60% in men over the age of 65 years (Rawla, 2019). International mortality rates for prostate cancer vary considerably

worldwide, in 2018 highest mortality rates were recorded in Central America (10.7 per 100,000 people), followed by Australia and New Zealand (10.2) and Western Europe (10.1). The lowest rate was reported in the countries of Asia (South-Central, 3.3; Eastern, 4.7 and South-Eastern, 5.4) and Northern Africa (5.8). One-third of the deaths for prostate cancer occurred in Asia (33.0%, 118,427 of deaths), followed by Europe (29.9%, 107,315 of deaths). The mortality rate of prostate cancer rises with age, and almost 55% of all deaths occur after 65 years of age. (Rawla, 2019).

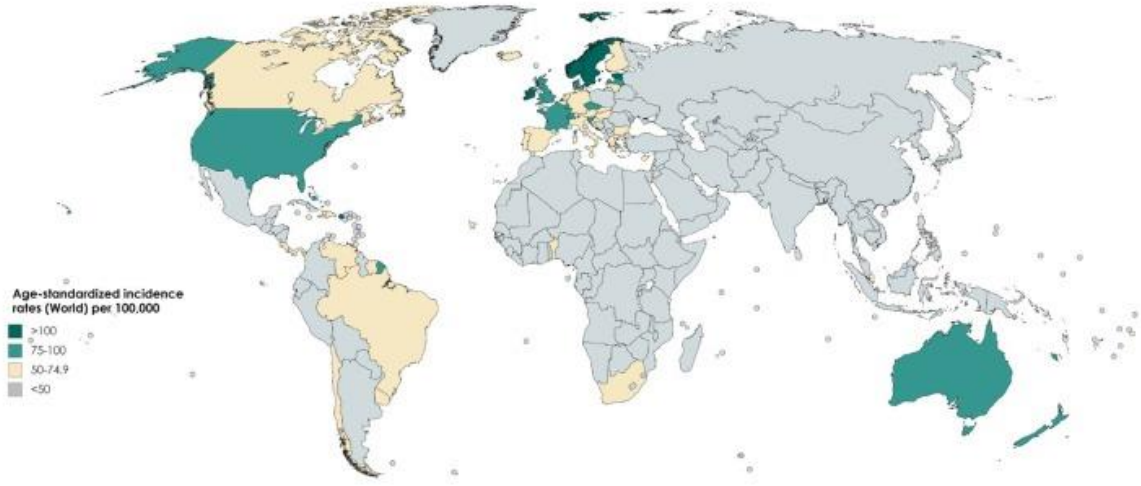

Figure 2.2 Map showing estimated age-standardized incidence rates for prostate cancer worldwide in 2018, in males including all ages (Ferlay, *et al*., 2019).

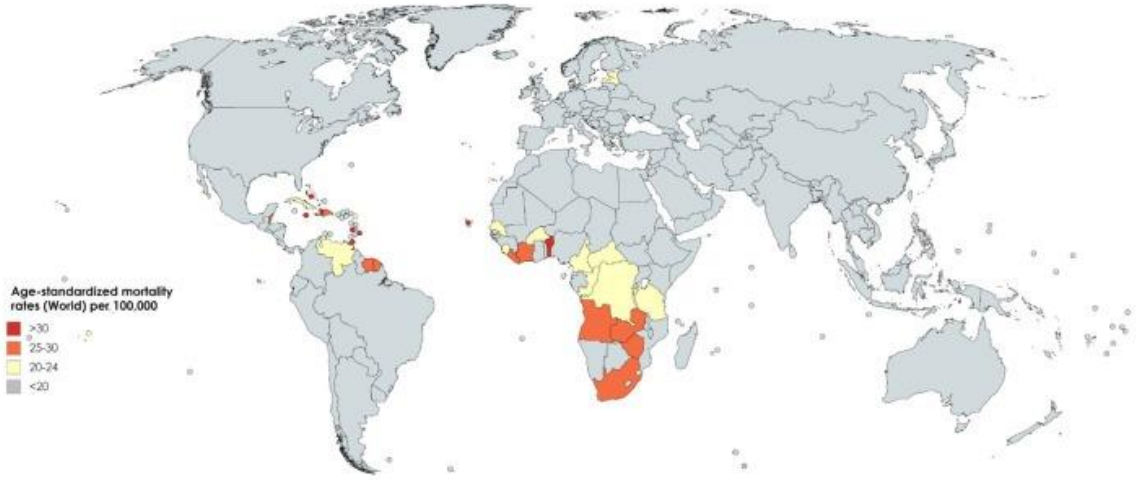

Figure 2.3 Map showing estimated age-standardized mortality rates for prostate cancer worldwide in 2018 in males including all ages (Ferlay, *et al*., 2019).

2.1.5.3.2 Etiology

The etiology of prostate cancer is the subject of numerous studies and remains largely unknown compared to other common cancers; however the clinical and experimental observations suggest that increased age, hormonal, genetic and environmental factors play a role in prostate cancer pathogenesis (Rawla, 2019).

2.1.5.3.3 Risk factors

It is becoming clear that its development involves a complex interplay of different factors including environment, culture, life style, and genetics. Environmental factors have detrimental influence on its development. Migration studies showed 5-fold increased risk among Japanese immigrants to US because of changed environment (Malik *et al*., 2018).

2.1.5.3.3.1 Age

Age is a well-established risk factor for prostate cancer; incidence of prostate cancer increases with age, prostate cancer is rare below the age of 40, age is an important risk factor in the development of PCa and its incidence rate increases with age especially after 50 years of age in White men who have no family history of the prostate cancer, and after 40 years of age in Black men or men with a familial history of prostate cancer (Seer, 2018), age-related trend is seen globally, both in developed and developing countries. PSA screening has led to an earlier age of prostate cancer detection as it has a lead time of approximately 10 years before any symptoms occur. Probability of developing prostate cancer increases from 0.005% in men younger than 39 years of age to 2.2% in men between 40 and 59 years, and 13.7% in men between 60 and 79 years (Ng, 2021).

2.1.5.3.3.2 Cigarette smoking

Cigarette smoking was another potential risk factor for its development owing to a number of reasons. Smoking may be linked with disease because of hormonal changes. Smokers had increased level of testosterone and androsterone and may be involved in cancer progression. Recently a meta-analysis was conducted that showed strong relationship of smoking with its onset and progression, it also showed that smoking was associated with mortality and incidence of disease (Li, *et al*., 2012).

2.1.5.3.3.3 Hormonal factor

Androgen receptor (AR) is considered the main driver in PCa growth and progression and most drugs are directed against AR pathway. Once PCa spreads outside the prostate,

androgen deprivation therapy (ADT) represents the cornerstone of treatment in hormone-sensitive prostate cancer (HSPC) (Pisano *et al*., 2021). Research has found that people with high levels of IGF-1 have an increased risk of developing prostate cancer (Shanmugalingam, *et al*., 2016).

2.1.5.3.3.4 Genetic factor

About 20% of patients with prostate cancer report a family history, which may develop not only because of shared genes but also for a similar pattern of exposure to certain environmental carcinogens and common lifestyle habits. Several studies reported that inherited genetic background is associated with increased risk for prostate cancer, contributing to about 5% of disease risks. This risk is increased by several folds when high-penetrance genetic risk alleles are inherited (Rawla, 2019). Gene linkage studies reveal major susceptibility loci for prostate carcinoma on genes in seven different loci. Chromosome 1q24-25 that is referred as *HPC1* gene encodes the enzyme ribonuclease L (RNASEL) which play role in the innate immune defense mechanisms and the interferon (IFN)-mediated signaling, on analysis of human prostate cancer samples from patients with RNASEL mutations showed the presence of retrovirus unveiling the importance of antiviral defenses to prostate cancer development, detection of retroviral infections in some cases of prostate cancer also showed the potential connection of chronic retroviral infection and consequent tissue inflammation with cancer initiation. Another HPC gene (*HPC2/ELAC2*) was identified on chromosome 17p11 and encodes a protein with poorly understood function, ELAC2, which is involved in prostate cancer development by binding SMAD2 that up-regulate proliferation through activation of TGF-beta signaling pathway. The third identified HPC gene is macrophage scavenger receptor 1 (*MSR1*), which resides on chromosome 8p22. People who inherit faulty versions of BRCA 2 genes have an increased risk of developing different types of cancer. This includes prostate cancer. A recent study showed that your risk of developing prostate cancer is around 2 times higher than that of the general population if you have a faulty BRCA2 gene, risk of developing prostate cancer may also increase with faulty BRCA1 genes. But researchers need more studies to find out for sure (Li et al., 2022). X chromosome is also believed to have a role in prostate cancer inheritance because it contains the androgen receptor (AR) and because small deletions in Xq26.3-q27.3 region were noted in sporadic and

hereditary forms of prostate cancer. More recent studies in 301 hereditary prostate cancer affected families defined a number of other loci that may contribute to hereditary prostate cancer (Rawla, 2019), also several studies associated family history as risk factor for prostate cancer development; affected brother or father the risk in this situation become double and the risk were further increased, if more first degree relatives were suffering from it (Malik *et al*., 2018).

2.1.5.3.3.5 Ethnicity

Prevalence of prostate cancer highly varies among different racial groups, it is more common in Black men than in White men and least common in Asian men. There is a higher incidence, severity, and mortality rates amongst men of black African descent, highest incidence in USA amongst black men of African descent and mortality rates 2.4 times higher in black men in the USA when compared to white men. A review by Rani *et al.* explained the observations of lower TMPRSS-ERG fusion, PTEN deletion, differential methylation of genes (SNRPN, SHANK2, MST1R, and ABCG5), and up-regulation of MNX1 in men of African descent promoted ontogenesis due to the deletion of such protective tumor suppressor roles, another chemokines receptor, DARC, found in red blood cells where they remove chemokines from prostate tumor microenvironment, has been shown to be depleted in large proportion of African men, contributing to increased incidence and mortality rates in this ethnic group (Ng, 2021). Other factors positively associated with prostate cancer include diet; increased consumption of saturated animal fat and red meat, lower intake of fruits, vegetables, vitamins, and coffee, obesity (World Cancer Research, 2014) and physical inactivity, inflammation (Thapa, *et al*., 2015), hyperglycemia, infections, and environmental exposure to chemicals or ionizing radiation (Markozannes, *et al*., 2016).

2.1.5.3.4 Clinical features

Many prostate cancers are slow growing and may not have any harmful effects during a man's lifetime. Meanwhile, clinically significant cancers can cause problems such as blockage of the urinary tract, painful bone lesions and death (Drost *et al*., 2019), but aggressive form of prostatic carcinomas are able to metastasize (Mohammad, 2018). In prostate cancer patients diagnosed with local disease have a 99% 5-year survival rate; however, this 5-year survival rate drops to 28% in patients with metastatic disease (Harris

and Kerr, 2017). Advanced-stage prostate cancer (PCa) patients are often diagnosed with bone metastases which remain incurable. PCa cells prevalently cause osteoblastic lesions characterized by an excess of bone formation (Hensel and Thalmann, 2016). If prostate cancer has already spread to other parts of the body (advanced or metastatic prostate cancer), it can cause symptoms such as: back or bone pain that doesn't go away with rest, tiredness, weight loss for no reason (National Institute for Health and Care Excellence (NICE), 2019).

2.1.5.3.5 Diagnosis of prostate cancer

Prostate cancer (PCa) has traditionally been diagnosed by digital rectal examination (DRE) and prostate-specific antigen (PSA) blood test, followed by trans rectal ultrasound (TRUS) guided biopsy. Abnormal results with either test may be due to benign prostatic enlargement (BPH) or infection, rather than cancer (Descotes, *et al*., 2019).

2.5.3.5.1 Prostate Specific Antigen (PSA)

PSA is an organ specific protein, and therefore can be elevated in non-malignant prostate pathologies such as benign prostatic hyperplasia (BPH) and Prostatitis, clinically, PCa is suspected on the basis of abnormal digital rectal examination (DRE) and/or elevated PSA levels, initial prostate investigations of prostate begin with measuring serum PSA level (Law *et al*., 2020). Total PSA (tPSA) remains the cornerstone of biological and landscape of tumor markers, as reported in a recent exhaustive review (Lamy, *et al*., 2017).

2.1.5.3.5.2 Digital Rectal Examination (DRE)

Rectal examination is carried out in a systematic way to evaluate voiding dysfunction in male; its performance for initial detection of cancer is limited, most patients detected with PCa during screening PSA program have normal DRE. However, palpation of irregularity or nodule during DRE still remains an indication for prostate biopsy regardless of the level of PSA. DRE may not significantly reduce mortality, but instead may result in a high number of false-positives leading to unnecessary invasive diagnostic tests that can precipitate pain, erectile dysfunction, and urinary incontinence, as well as over diagnosis and overtreatment of prostate cancer (Cui *et al*., 2016).

2.1.5.3.5.3 Prostate Biopsy

Prostate biopsy is the gold standard diagnostic technique for the detection of prostate cancer; the prostate gland tissue is taken out with needle or during surgery to check if

there is cancer or any abnormal cells in the prostate gland, diagnosis of prostate cancer cannot be made without a biopsy (Morgan, *et al*., 2017,Streicher *et al*., 2019).

2.1.5.3.5.4 Magnetic Resonance Imaging (MRI)

Imaging is becoming critical for guiding management decisions in prostate cancer (PCa) both at initial diagnosis and at recurrence. Multipara metric magnetic resonance imaging and positron emission tomography of the prostate have proven valuable in the detection and localization of aggressive disease, recent reviews have suggested that contemporary multi-parametric MRI reliably detects clinically significant prostate tumors and provides critical information regarding tumor location, volume, grade and stage, (Thompson *et al*., 2013, Ludwig, *et al*., 2018).

2.1.5.3.5.5 Bone scan

The bone scan index is a method to quantitatively measure the burden of bony disease, and can assess both disease progression and regression (Mota *et al*., 2019). Whole-body diffusion-weighted MRI and molecular imaging will play increasing roles in defining the presence and extent of metastatic disease, enabling assessment of treatment response and disease progression, and will improve our understanding of disease biology (Perez, *et al*., 2019).

2.1.5.3.6 Gleason grading

The Gleason grading system is one of the most reliable methods for evaluating prostate cancer aggression, developed in 1967 and updated in 2014. Gleason grades are used for describing prostate adenocarcinoma growth patterns, and they are related to disease severity. According to this system, prostate cancers are scaled into five grades based on glandular patterns of differentiation. It varies from1-5; grade 1 is excellent prognosis, while grade 5 is poor prognosis (Linkon *et al*., 2021).

2.1.5.3.7 Treatment and management

Definitive treatment for localized disease includes radical prostatectomy and/or radiotherapy. Radiotherapy can also be combined with Androgen deprivation therapy (ADT) for high risk patients. Patients who have disease progression (both metastatic and non-metastatic) on effective ADT with testosterone < 50 ng/dL are considered castration resistant (CRPC) and require next-generation endocrine agents to suppress their cancer (Barsouk *et al*., 2020). ADT is beneficial for unfavorable intermediate-risk prostate

cancer patients receiving curative radiotherapy. However, for favorable IR patients the latest NCCN guideline recommends RT alone (Amit, *et al*., 2019).

2.2 Hemostasis

Hemostasis defined as a complex biological pathway aimed at arresting blood leakage from injured venous and arterial vessels, and concomitantly preventing excessive or unwarranted blood clotting when these structures are undamaged (Lippi and Favaloro, 2018).There are five different components involved: blood vessels, platelets, plasma coagulation factors and their inhibitors and the fibrinolytic system (Bain, *et al*., 2011).

2.2.1 Platelets

Are small fragments of cytoplasm derived from megakaryocytes (Bain, *et al*., 2011).

2.2.1.1Plateletsproduction

Platelets are small a nucleate cells derived from the cytoplasmic fragmentation of their megakaryocytes (MK) precursor. MKs produced in the bone marrow through a highly orchestrated process. HSCs give rise to progenitors which progressively commit to the megakaryocytic lineage to produce immature MKs. MK maturation involves an increase in DNA content (up to 64N) through endomitosis accompanied by massive enlargement of the cytoplasm, the emergence of numerous alpha and dense granules and the development of an extensive membrane network, the demarcation membrane system (Hoffbrand,2019). Terminally differentiated MKs intimately associated with the sinusoidal endothelium of the bone marrow. Following extensive cytoskeletal remodeling, fully mature MKs extend cytoplasmic projections called pro platelets into the vessel lumen, where platelets are released under shear forces produced by the circulating blood (Strassel *et al*., 2018).

2.2.1.2 Platelet structure

Platelets are small discoid cell fragments. On average they are 1.5-3.5 um in diameter. They do not contain a nucleus and are bounded by a typical lipid bilayer membrane (Bain, *et al*., 2011). Glycoproteins Ib and IIb/IIIa are important in the attachment of platelets to von Willebrand factor and hence to vascular sub endothelium where metabolic interactions occur, binding site for IIb/IIIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation. The plasma membrane invaginates into the platelet interior to form an open membrane system which provides a large

reactive surface to which the plasma coagulation proteins selectively absorbed. The membrane phospholipids are important in the conversion of factor X to Xa and prothrombin thrombin (Hoffbrand,2016). The platelet contains three types of storage granules: dense, alpha and lysosomes. Alpha granules contain fibrinogen, VWF, beta thromboglobulin and other clotting factor. Dense granules contain ADP, ATP and calcium. Lysosomes contain hydrolytic enzymes and peroxisomes contain catalase. During the release reaction, the contents of the granules are discharged into the open canalicular system. Platelets are also rich in signalling and cytoskeletal proteins which support the rapid switch from quiescent to activation that follows vessel damage (Hoffbrand, 2019).

2.2.1.3 Platelet function

Platelets maintain hemostasis by adhering to the vascular endothelium, aggregating with other platelets, and initiating the coagulation cascade, leading to the production of a fibrin mesh, which effectively prevents significant blood loss. Platelets are also crucial in inflammation, tissue growth, and immune response. These processes are under the mediation of the release of compounds from the alpha and dense granules, which include numerous growth factors as well as IgG and components of the complement system (Fountain and Lappin, 2021).

2.2.1.4 Platelet plug formation

Platelets can become activated in response to exposed collagen, thrombin, ADP, or other compounds. In response to tissue injury, exposed collagen on the sub endothelial surface can bind directly to either the platelet or to vWF. The vWF is a molecule that can bind to both collagen and the platelet, via the GPIb receptor on the platelet surface. As a result, vWF acts as a bridge, forming a complex of collagen, vWF and platelet, which results in adhesion to the vascular surface. Also exposed collagen can bind directly to the platelet by binding to the GPVI receptor. Binding to the GPIb and GPVI receptors causes activation of the platelet and beginning intracellular signaling cascades, result in the release of both alpha and dense granules, as well as activation of other enzymes, such as cyclooxygenase-1, which synthesizes thromboxane A2 (TXA). The release of alpha and dense granules is crucial for the recruitment of nearby platelets and further activation of the platelet, as a result of degranulation ADP, TXA, serotonin, fibrinogen, and P-Selectin

are secreted into the plasma (Hoffbrand, 2016). ADP and TXA are important in the activation of platelets, released ADP binds to P2Y and P2Y receptors on the platelet surface, further increasing signal transduction and activation in the platelet, while TXA binds to thromboxane prostanoid receptors, increasing activation of nearby platelets. Both of these are critical in the recruitment of other platelets to form a large platelet plug. Serotonin acts in a similar, but less potent, way on 5HT receptors (Elsayed, 2015). Normal platelets count is 150 to 450 $X10^9$ cell/L, with normal life span ranges from 7 to 10 days and about one third of platelets sequestrate in the spleen (Hoffbrand, 2019).

2.2.2 Platelets Estimation

2.2.2.1 Platelets and Platelet Indices (PI)

PLT indices are a group of parameters that are used to measure the total amount of PLTs, PLTs morphology and proliferation kinetics, include PLT count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) (Zhang *et al*., 2015), and platelet large cell ratio (Bain, *et al*., 2011), most frequently evaluated parameters are mean platelet volume (MPV), platelet diversity index (PDW), plateletcrit (PCT) and the presence of larger platelets (P-LCRs platelet larger cell ratio),The measurement of PIs does not generate additional costs and can be performed during routine cell blood count, not requiring additional blood samples (Pogorzelska *et al*., 2020).

2.2.2.2 Mean Platelet Volume (MPV)

MPV reflects the size of platelets, which is related to platelet production and activation, normally ranges between 7.5 and 12.0 fl inversely proportional to the platelet count; this means that the increased production of platelets is accompanied by a reduction in their mean volume (Wu *et al*., 2019b). MPV measures the average size of the platelet and it is directly derived from the analysis of the platelet distribution curve (Nishimura, *et al*., 2021). Elevated MPV correlates with increased platelet aggregation, enhanced synthesis, and release of thromboxane TXA2 and *β*-thromboglobulin (Korniluk *et al*., 2019).

2.2.2.3 Platelet Distribution Width (PDW)

Platelets distribution width evaluate platelet anisocytosis and the plateletcrit, which is the volume of circulating platelets in a unit volume of blood, it is directly measures variability in platelet size and changes occurring with platelet activation and suggests the heterogeneity in platelet morphology (Nishimura, *et al*., 2021).

2.2.2.4 Platelet Large Cell Ratio (P-LCR)

Platelet Large Cell Ratio (P-LCR) also known as Large Platelet Parameter and Platelet Large Cell Count (P-LCC) calculated by some automated blood counter instruments, reflects the number of platelets falling above the 12fl threshold on the platelet size histogram divided by the total number of platelets (Bain, *et al*., 2011). Platelet large cell ratio (PLCR) represents the proportion of large platelets which reflects alteration of platelet dimension, with inverse correlation to PLT and direct correlation with MPV and PDW (Nickavar and Sadeghi-Bojd, 2020).

2.2.2.5 Plateletcrit (PCT)

Plateletcrit provides complete information on total platelet mass and shows percentage of blood occupied by PLT (Akpinar *et al*., 2014, Michalak *et al*., 2021), PCT is useful for detecting platelet quantitative abnormalities, normal range for PCT is 0.22-0.24 % (Nishimura, *et al*., 2021).

2.2.3 Platelets Lymphocytes Ratio

There are known several serum biomarkers and hematological indices of inflammation such as: CRP, fibrinogen, lymphocyte-monocyte ratio (LMR), and platelet-lymphocyte ratio (PLR) (Huszno *et al*., 2022). PLR has been used as a biomarker for differentiating between two or more disorders or as a predictor of multiple pathological conditions as inflammatory disease and cancer (Soliman et al., 2020), PLR reported a strong link between prostate cancer and inflammation; inflammation impacts every step of tumorigenesis, such as tumor initiation, promotion and metastatic progression. Low lymphocyte counts are reported to be an adverse prognostic marker for many diseases, including prostate cancer, while increased platelet counts have been associated with poor prognosis and tumor load in patients with cancer (Murray *et al*., 2020).

PLR is calculated as platelet count divided by lymphocyte count **(**Cuenco, 2020).

platelet-to-lymphocyte ratio have been studies as a novel inflammatory marker and prognostic markers for cardiovascular disease, inflammatory disorders and malignancies. The two circulating biomarkers which are calculated using the peripheral blood cells, platelet to lymphocyte ratio, has obtained prognostic significance in a variety of malignances, as well as PCa. A large amount of studies have examined the prognostic

role of this marker in PCa patients, however, the importance and consistence are still needed to be determined (Guo *et al*., 2018).

2.2.4 Blood coagulation

Once the platelet plug has been formed by the platelets, the clotting factors are activated in a sequence of events known as coagulation cascade which leads to the formation of Fibrin from inactive fibrinogen plasma protein, thus fibrin mesh is produced all around the platelet plug to hold it in place this step known as secondary hemostasis; however, without this process the healing of a wound would not be possible (Elsayed, 2015).

2.2.4.1 Fibrinogen

Fibrinogen is an important indicator of the coagulation system, also it is an acute-phase protein that is mainly synthesized by hepatocytes and converted into insoluble fibrin by activated thrombin, Fibrinogen plasma level increases in some clinical conditions such as malignancy and systemic inflammation, when fibrinogen levels increased, it is deemed to be an unfavorable prognostic marker as in some malignancies, such as those of the digestive system, gynecologic malignancies, urologic neoplasms, and soft tissue sarcomas (Wen *et al*., 2015).

2.2.4.2 D-dimer

D-dimer molecules are generated through the degradation of cross-linked fibrin during fibrinolysis. D-dimer generation requires the activity of three enzymes: thrombin, factor XIIIa, and plasmin; process starts when thrombin generated by the coagulation system converts soluble fibrinogen to fibrin monomers, these monomers then form fibrin polymers through noncovalent interactions based on allosteric changes within the protein as a result of thrombin cleavage of fibrinopeptides from the N-terminal domain. Fibrin is strengthened through interactions with factor XIII and after the activation by thrombin, cross-links the D domains of adjacent fibrin monomers. Plasmin digestion of the fibrin clot results in the D-dimer molecule (Johnson *et al*., 2019).

D-dimer is a degradation product of fibrin which is produced by plasmin-induced fibrinolytic activity, considered as gold standard among markers and biomarker that indicate measured in plasma or whole blood that reflect activation of hemostasis and fibrinolysis (Undas, 2020). D-dimer is a soluble (FDP) that results from the systematic degradation of vascular thrombi through the fibrinolytic mechanism. Because of this, it

serves as a valuable marker of activation of coagulation and fibrinolysis in a number of clinical scenarios. Most commonly, D-dimer has been extensively investigated for excluding the diagnosis of venous thromboembolism (VTE) and is used routinely for this indication. In addition, D-dimer has been evaluated for determining the optimal duration of anticoagulation in VTE patients, for diagnosing and monitoring disseminated intravascular coagulation, and for monitoring other conditions in which the patient is at high risk of bleeding or thrombosis (Johnson *et al*., 2019).

Elevated plasma D-dimer levels are seen in patients with various types of cancer and solid tumor such as prostate cancer, cervix (Sun *et al*., 2015) and esophageal squamous cells (Feng *et al*., 2016), because procoagulant factors lead to constitutive activation of the coagulation cascade, which results in thrombin generation followed by fibrin formation (Lee *et al*., 2017).

2.2.5 Hematological markers and prostate cancer risk

Risk factors for prostate cancer are not well understood. Red blood cell, platelet and white blood cell indices may be markers of a range of exposures that might be related to prostate cancer risk. Combination of several biomarkers for early detection may lead to enhanced sensitivities and specificities. The mechanisms underlying the association of MPV and PDW with PCa are currently unclear, numerous studies have identified enhanced platelet activation occurred in PCa, because platelet-derived growth factor proteins are potent stimulators of cell proliferation/transformation in PCa, for that High expression of PDGF alpha-receptor activation is associated with bone metastases in castration-resistant PCa (Russell *et al*., 2010). Large prospective study designed in UK by Watts et al included more than 200,000 men observed that several hematological parameters and blood indices with prostate cancer risk and mortality implicate shared common causes with type of cancer, also he found that a higher platelet count was associated with an increased risk of prostate cancer diagnosis, but platelet indices were not associated with prostate cancer mortality (Watts *et al*., 2020). Also other study suggests that combined use of PSA, MPV, and PDW can be used as a surrogate for the presence of PCa (Fu *et al*., 2018), PLR significantly increase in PCa patients with bone metastases and are valuable in the diagnosis of bone metastases in PCa patients (Zhang *et al*., 2019). PLR gives promising value as a systemic inflammatory marker in predicting

prostate cancer in suspected patients (Adhyatma and Warli, 2019). Platelets play an important role in tumor growth and metastasis through tumor cell-induced platelet aggregation (TCIPA), ability of cancer cells to induce platelet aggregation tends to correlate with their metastatic potential. Reciprocally, platelet- induced signaling within cancer cells can further enhance their invasive potential, including mediating epithelial to mesenchymal transition (Rudzinski *et al*., 2020).

The main risk factors for coagulation activation and thrombosis are malignancy and older age, thrombosis risk may be associated with increased level of coagulation markers such as fibrinogen and D-dimer. These disorders contribute to mortality and morbidity of PCa patients and are correlated with bone metastasis in 91% of the patients (Al Saleh et al., 2018). Disseminated intravascular coagulation (DIC) is the most frequent coagulation disorder associated with metastatic prostate adenocarcinoma (Palma Anselmo *et al*., 2016). In prostate cancer, the incidence of DIC complication is from 13% to 30% whereas that of bleeding symptoms is only 0.40% to 1.65% (Wada *et al*., 2012).

2.3 Previous Studies

A study conducted at Harbin Medical University, China by Fu *et al*., (2018), the study showed that PCa patients had reduced MPV and elevated PDW compared to BPH patients(p-value <0.001) (Fu *et al*., 2018).

A retrospective study at Shandong University, Jinan, China, found that MPV was significantly less in PCa than non-PCa among men (P = 0.001). On the other hand, PDW was significantly increased in PCa than non-PCa among men. The platelet count among men with PCa tended to be more than that of the non-PCa study group, but there was no statistical difference (P = 0.09) (Song *et al*., 2022).

A study conducted at Sumatera Utara University, Medan, Indonesia by Adhyatma and Warli (2019). Included 298 patients; 126 (42.3%) BPH and 172 PCa, there result showed a significant difference for PLR values between 2 groups, PLR was higher in patients with prostate cancer, $p < 0.05$ (Adhyatma and Warli, 2019).

Pooled analysis at China–Japan Friendship Hospital, Beijing, China performed by (Guo *et al*., 2018), demonstrated a significant elevation in PLR in prostate cancer patients and it predicts poor overall survival, P-value 0.001 (Guo *et al*., 2018).

A study at University of Health Sciences, Adana City Teaching and Research Hospital, Adana, Turkey by (Börekoğlu *et al*., 2020), the study demonstrated that there was no statistically significant difference between the patients with prostate cancer and patients with benign conditions according to the platelet counts, MPV and PDW and PCT. Additionally, there were no statistically significant differences between the groups regarding the PLR (Börekoğlu *et al*., 2020).

A study conducted at Bezmialem Vakif University, Istanbul, Turkey by Kalkan and Caliskan in (2019), reveal that there was a statistically significant difference for D-dimer between patients with prostate cancer and patients with BPH, D-dimer levels were higher than in patients with BPH with P-value < 0.05 (Kalkan and Caliskan, 2020).
A case control study at Odessa National Medical University, Odessa, Ukraine conducted by Tarabrin *et al*., (2019), showed that the D-dimer level was higher in patients with prostate cancer than those with benign prostatic hyperplasia (Tarabrin *et al*., 2019).

A retrospective study conducted at Sakarya University Adapazari, Sakarya, Turkey was performed by Saglam and Cimen (2021), found significant elevation in D-dimer levels in patients with prostate cancer compared to patients with benign prostatic hyperplasia with P-value 0.011 (Saglam and Cimen, 2021).

A prospective study was performed at Seoul National University Bundang Hospital, Seongnam, Korea by Hong *et al*., (2010), demonstrated that the cancer group had significantly higher plasma D-dimer levels than did the control group with $P = 0.007$ and $P = 0.018$, respectively (Hong *et al*., 2010).

A prospective study conducted at Hitit University, Çorum Training and Research Hospital, Çorum, Turkey by Çalışkan and Sunger (2017), reported that plasma D-dimer level was higher in patients with prostate cancer (Caliskan and Sungur, 2017).

A study conducted in Beijing, China by Wang and Xing, (2021). Involved A total of 423 pathologically diagnosed patients with PCa. The study demonstrated that plasma level of D-dimer was significantly high in prostate cancer patients (p-value < 0.001) (Wang and Xing, 2021).

CHAPTER III

Material & Methods

Chapter III

3. Material and Methods

3.1 Study Design

This study was hospital base, case control study.

3.2 Study Area

This study was conducted at Khartoum. hospital, Khartoum – Sudan.

3.3 Study Duration

This study was conducted during the period from April to October 2022.

3.4 Study Population

The study population was Sudanese patients of prostate cancer whom refereed to Khartoum hospital and accepted to participate in the study.

3.4.1 Inclusion Criteria

Sudanese men with prostate cancer patients from all age were included.

3.4.2 Exclusion Criteria

Other types of cancer patients, prostate cancer patients who refused to participate in this study and patients with known hemostatic disease were excluded.

3.4.3 Estimated Sample Size

According to the following equation samples were collected for cases and controls.

$$N= (Z^2 \times P (1-P) / e^2$$

Where Z = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI).

P is expected true proportion.

e is desired precision.

$= 1.96^2 \times 0.099 \times (1-0.099) / 0.05$

= 137

Due to high cost of estimated parameters the sample size was 100.

3.5 Ethical Considerations

The ethical approval was obtained from Sudan University of Science and Technology, College of Medical Laboratory Research Board and Khartoum hospital. After explains the purpose of the research with simple and clear words for the participant's, they were

told that they have rights to voluntary, they signed informed consent and they can withdraw at any time without any deprivation. All participants have rights to no harm (privacy and confidentiality) by using coded questionnaire and the remaining samples were not be reused for other research and the data will be secured. All participants' has rights to benefit from the researcher knowledge and skills about prostate cancer.

3.6 Sampling technique

Non probability convenience sampling technique was used.

3.7 Data collection

Data were collected using a pre-structured questionnaire filed by face-to-face interview with participants.

3.8 blood collection and Processing:

Venous blood was collected from each participant and distributed in two containers, 2.5 ml in EDTA containers for complete blood count test and 1.8 ml in trisodium citrate containers for measuring D.dimer level. The EDTA samples were analysed immediately while trisodium citrate samples were centrifuged at 4000 rpm for 15 min to obtain the PPP, then the PPP kept at – 70^{o}C until analysis.

3.9 Blood count

Complete blood count was measured using automated blood counter (Sysmex X N1000) .

3.9.1 Principle of the analyzer

This instrument enables quantitative, identification and existence ratio analysis and flagging of tangible components of blood and body fluid (red blood cells, white blood cells, platelets and other cells) by means of electrical impedance, laser light scattering and dye bonding. (Sysmex cooperation, 2014).

3.9.2 Procedure of CBC assay

Blood sample after has been mixed thoroughly, placed in the tube holder and then press the start switch on the analyzer and aspiration began. Once the analysis finishes the result appeared on the display screen and printed out.

3.9.3 Quality Control of Sysmex

The reliability of this instrument and reagents was monitored by using control materials XN Check (three levels) for the calibration of the instrument for WBC, RBC, HBG, PLT and RET. and XN Check BF (two levels) for the calibration of the instrument for PLT-F

(platelet count obtained from the PLT-F channel), to monitor an instrument performance before analyzing sample (Sysmex cooperation, 2014).

3.10 Calculation of Platelets to Lymphocytes Ratio (PLR)

PLR was calculated as the platelet count divided by the lymphocyte count (shen, *et al*., 2019). Normal range : 36.63-149.13 (Wu *et al*., 2019a).

3.11 Estimation of plasma D- Dimer level

D.dimer was measured using Ichroma^{TM} D.dimer.

3.11.1 Principle of D. Dimer assay

The test uses the sandwich immune-detection method, such that the detector antibodies in buffer bind to antigen in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized-antibodies on the test strip. The more antigens in the plasma will form more antigen-antibody complexes which lead to stronger fluorescence Signal by detector antibodies, which is processed by instrument for Ichroma^{TMD} tests to show D-Dimer concentration in the sample.

3.11.2 Procedure of D. Dimer assay

The test reagent brought to room temperature, then 10 ul of sample transferred to a tube containing the detection buffer and mixed thoroughly. 75 ul of the sample mixture pipetted out and loaded into sample well on the cartridge and incubated at room temperature for 12 minutes, then inserted into the cartridge holder of the instrument and scanned then the result appeared on the display screen and recorded.

3.11.3 Quality Control of Ichroma^{TMD}

Two methods were performed to ensure the reliability of the instrument. First system check is performed to confirm the ichroma ^{TM} works properly. And second by using control material universal control 1(Boditech Med incorporated, 2018).

3.12 Data analysis

Data were analyzed using statistical package for social science software (SPSS version .25), and presented in form of tables and figures. The statistical analysis was performed by using independent t-test and chi-square test (A P-value of < 0.05 was considered significant).

CHAPTER IV

Results

Chapter IV

4. Results

4.1 Description of the study population

This study was conducted in Khartoum state in the period from April to October 2022. One hundred individuals were included in this study; 50 patients with prostate cancer as case and 50 healthy volunteers as control were matching in age and sex, age ranging from 50 to 85 years old with mean age (67.14±7.3) years in case and control. D-dimer, platelets count and indices and platelet lymphocyte ratio (PLR) were measured for all participants in this study.

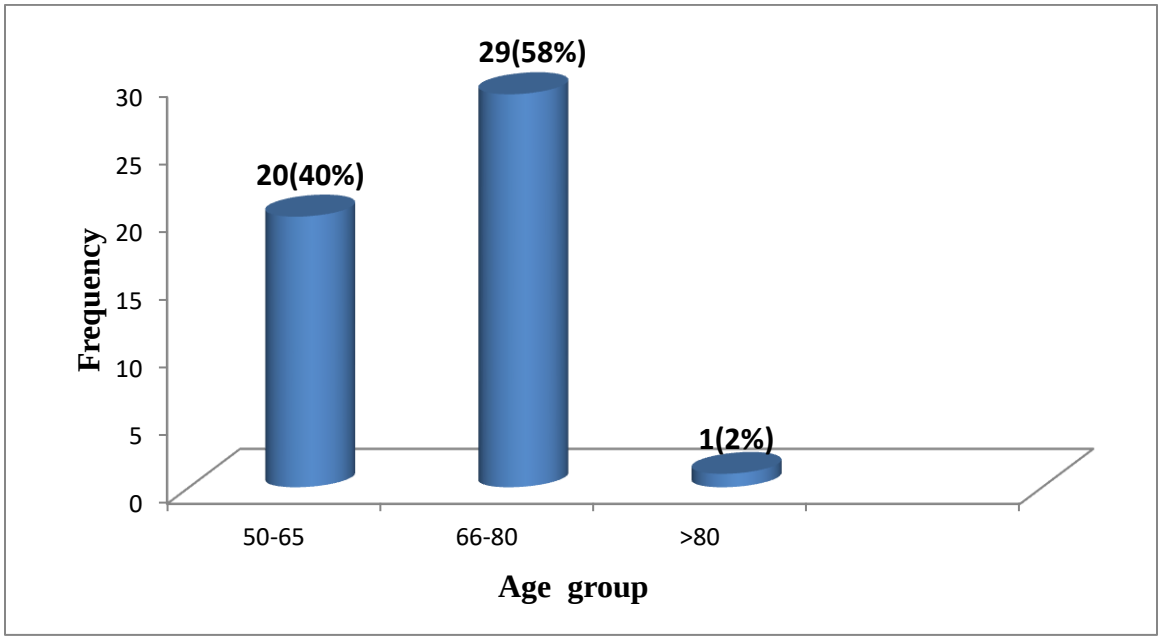

Figure: 4-1 Age distribution among study population

4.2 Comparison of D-dimer level between cases and controls

Patients with prostate cancer had significantly higher D-dimer level than control group *P-value*=(0.00) Table (4-1).

Table: 4-1 Comparison between case and control in mean of D-dimer level

Parameter	Mean± SD		*P-value*
	Case	Control	
D-Dimer	1.72±2.4	0.18±0.07	0.00

4.3 Results of Hematological parameters

The means of platelets count, mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR), plateletcrit (PCT) and platelet lymphocyte ratio (PLR) in case and control were (259±111.83× 10^3/µl) Vs. (251.46±58.98×10^3/µl), (10.20±0.88 fl) Vs. (8.06±0.81 fl), (11.81±2.06 fl) Vs. (10.49±1.04 fl), (26.61±7.15%) Vs. (14.64±5.17%), (0.26±0.10%) Vs. (0.19±0.04%) and (161.36±107.53) Vs. (159.34±66.39) respectively. The patients with prostate cancer had significantly higher MPV, PDW, PLCR, PCT (*P-value* = 0.00). Table (4-3). No significant differences was demonstrated with regard to platelets count and PLR (*P-value* > 0.05) . Table (4-2).

Table: 4-2 Comparison of hematological parameters among study subjects

Parameter	Mean± SD		*P-value*
	Case	Control	
PLT (X10^9/L)	259.46±111.83	251.46±58.98	0.6
MPV(FL)	10.20±0.88	8.06±0.81	0.00
PDW(FL)	11.81±2.06	10.49±1.04	0.00
PLCR(%)	26.61±7.15	14.64±5.17	0.00
PCT(%)	0.26±0.10	0.19±0.04	0.00
PLR	161.36±107.53	159.34±66.39	0.9

4.4 Frequency of chemotherapy among cases

There were twenty seven (54%) of prostate cancer patients have received chemotherapy whereas 23 (46%) have not received chemotherapy, as shown in figure (4-2).

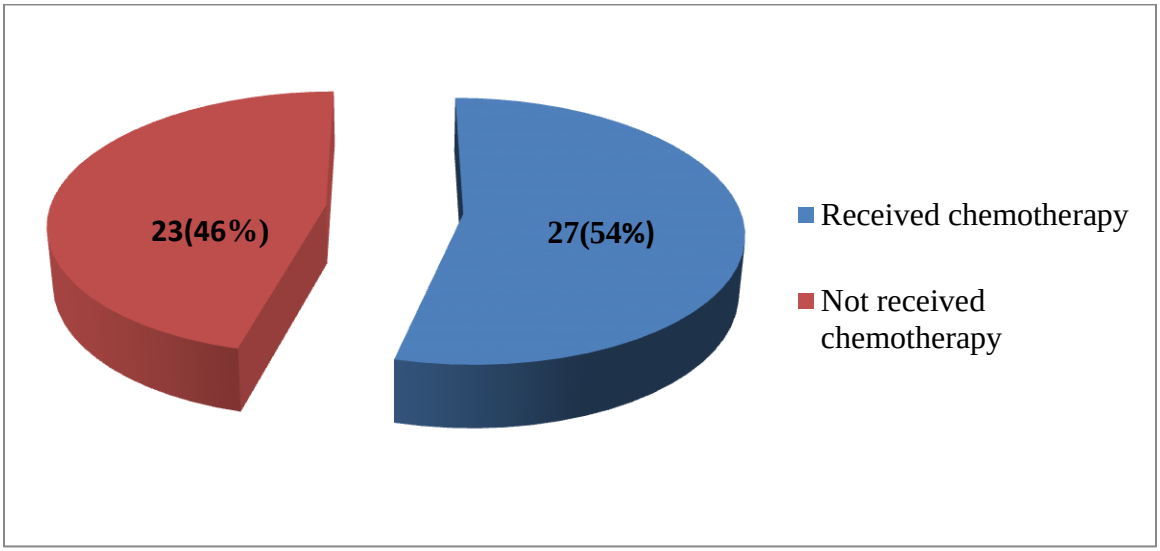

Figure: 4-2 Frequency of chemotherapy among Prostate cancer patients

4.5 Association between means of hematological parameters and chemotherapy

Result showed that Patients receiving chemotherapy had higher PDW than patients were not *P-value* = (0.05). Result also showed no significant difference in D-Dimer, PLT, MPV, PLCR, PCT and PLR in relation to chemotherapy *P-value* >0.05). Table (4-3).

Table: 4-3 Association between means of hematological parameters and chemotherapy

Parameter	chemotherapy		*P-value*
	Received chemotherapy	Not received chemotherapy	
PLT	233.96±111.01	289.39±107.51	0.08
MPV	10.40±0.92	9.98±0.81	0.09
PDW	12.32±2.23	11.20±1.68	0.05
PLCR	28.14±7.40	24.81±6.56	0.09
PCT	0.23±0.10	0.28±0.10	0.1
D-Dimer	1.87±2.68	1.55±2.26	0.6
PLR	167.25±119.0	154.43±94.49	0.6

4.6 Frequency of metastasis among case group

Among this study eleven of prostate cancer patients(22%) were with metastasis whereas 39 (78%) without metastasis as shown Figure (4-3).

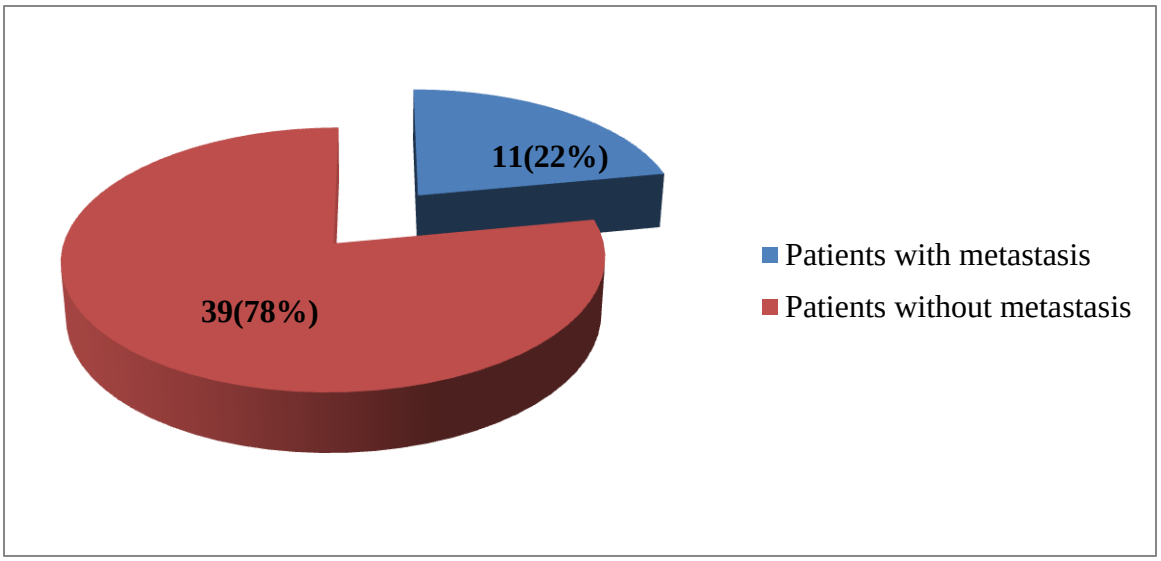

Figure: 4-3 Frequency of metastasis among case group

4.7 Association between means of hematological parameters and metastasis

The result showed no significant statistical difference between mean of hematological parameters among metastatic patients *P-value* > 0.05 as in Table (4-4).

Table: 4-4 Association between means of hematological parameters and metastasis

Parameter	Metastasis		*P-value*
	Patients with metastasis	Patients without metastasis	
PLT	250.80±99.76	261.62±115.73	0.7
MPV	10.18±1.16	10.21±0.82	0.9
PDW	12.21±2.62	11.71±1.92	0.5
PLCR	26.77±8.99	26.57±6.75	0.9
PCT	0.25±0.09	0.26±0.10	0.7
D-Dimer	2.40±3.11	1.55±2.31	0.3
PLR	171.42±118.70	158.84±106.04	0.7

CHAPTER V

Discussion, Conclusion & Recommendations

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5. Discussion, Conclusion and Recommendation

5.1 Discussion

Prostate cancer is the second most commonly diagnosed male malignancy and the 5th leading cause of cancer death in men worldwide (Rawla, 2019). In Sudan, in males the prostate cancer is on top of the incidence (Maki, 2022).

In this study one hundred individuals were included; 50 prostate cancer patients (cases) and 50 healthy volunteers (controls) were matching in age and sex. Age ranging from 50 to 85 years old with mean age (67.14±7.3) years in case and control, A similar result was also shown in study in Indonesia conducted by Adhyatma and Warli (2019), they reported that the mean age of prostate cancer patients was 67.99 ± 7.48 years old.

D-dimer is one of the fibrin degradation products and the level of D-dimer is a result of fibrinolysis activation. Elevated plasma D-dimer levels are seen in patients with various cancer types, because procoagulant factors lead to activation of the coagulation cascade result in thrombin generation followed by fibrin formation (Caliskan and Sungur, 2017).

This study showed that D-dimer levels were higher in patients with PCa than in control group with p-value (0.00). This is in line with (Kalkan and Caliskan, 2020) in Turkey, who stated that D-dimer level was significantly higher in patients with prostate cancer compared to patients with benign prostatic hyperplasia. This also supported by the study of Tarabrin *et al*., (2019), who reported that levels of D-dimer increase significantly in patients with prostate cancer. The authors from Turkey found significant elevation in D-dimer levels in patients with prostate cancer compared to patients with BPH, Also agrees with study in Korea by Hong *et al*., (2010), they found that prostate cancer group had significantly higher plasma D-dimer levels than control.

Recent investigations showed that platelets play critical roles in mediating cancer cells growth, dissemination, and angiogenesis (Sharma *et al*., 2014). Activated platelets are associated with cancer progression and metastasis (Goubran *et al*., 2014)

The hematological results in our study revealed that patients had significantly higher mean platelet volume, platelet distribution width, platelet large cell ratio and plateletcrit than control with (p-value = 0.00). This is compatible with two studies conducted in China by Fu *et al*., (2018) and (Song *et al*., 2022) who reported that patients with PCa

had an elevated PDW, and disagrees with (Fu *et al*., 2018) and (Song *et al*., 2022), they reported that the MPV was reduced in PCa patients, also disagrees with (Börekoğlu *et al*., 2020) in Turkey, reported that there was no significant difference between PCa patients and patients with benign conditions according to the MPV, PDW and PCT.

Current study showed that PCa patients had higher platelet count and PLR than control group, but there was no statistical difference. This result agrees with (Börekoğlu *et al*., 2020), they reported that there was no significant difference between the patients with prostate cancer and patients with benign conditions according to the platelet counts, also consistent with (Börekoğlu *et al*., 2020), they found that there was no significant difference between the patients with prostate cancer and patients with benign conditions regarding the PLR, and also disagrees with study in Indonesia conducted by Adhyatma and Warli (2019), they reported that the PLR was higher in patients with prostate cancer. This study also disagrees with (Guo *et al*., 2018) in China, they found that the patients with prostate cancer had a higher PLR.

Current study revealed that patients receiving chemotherapy had higher PDW than patients were not (*P-value* = 0.05), and showed no significant difference in D-Dimer, platelets indices and PLR in relation to chemotherapy (*P-value* >0.05). This is in line with (Başeskioğlu et al., 2019) who reported that there was no correlation between MPV and chemotherapy.

The present study also showed no significant statistical difference in mean of hematological parameters between metastatic and non-metastatic patients *P-value* > 0 .05, this finding is in line with (Hong et al 2010) in Korea, concluded that subjects with organ confined disease and those with an extra prostatic extension of a tumor had not demonstrated any significant differences in the preoperative D-dimer levels, and also disagrees with (Saglam and Cimen 2021) in Turkey, they reported that metastatic PCa had higher D-dimer values than non-metastatic PCa, and this findings also disagree with (Zhang *et al*., 2019) who reported that PLR significantly increase in PCa patients with bone metastases and is valuable in the diagnosis of bone metastases in PCa patients.

5.2 Conclusion

This study conclude that :

The D.dimer levels are higher in patients with prostate cancer. Also markers of platelets activation (MPV) and (PDW) were significantly increased in prostate cancer, prostate cancer patients had also higher PCT and PLCR. Furthermore, prostate cancer patients who receiving chemotherapy had higher PDW than patients were not receiving chemotherapy.

5.3 Recommendation

This study recommend

- D-dimer should be considered for patients with prostate cancer to detect signs of Disseminated intravascular coagulation.
- In order to get more informative data should use further investigations for prostate cancer patients include (protein C, protein S and thrombin generation tests).
- Further studies should be conducted using large sample size to obtain more accurate results.

References

Aaron, L., Franco, O.E. and Hayward, S.W. (2016). Review of prostate anatomy and embryology and the etiology of benign prostatic hyperplasia. *Urologic Clinics*, *43*(3), pp.279-288.

Adhyatma, K. P. & Warli, S. M. 2019. Diagnostic Value of Platelet–To-Lymphocyte Ratio in Prostate Cancer. *Open Access Macedonian Journal of Medical Sciences,* 7**,** 1093.

Akpinar, I., Sayin, M., Gursoy, Y., Karabag, T., Kucuk, E., Buyukuysal, M., Aydin, M. & Haznedaroglu, I. 2014. Plateletcrit. *Herz,* 39**,** 142-148.

Al Saleh, H. A., Haas-Neill, S., Al-Hashimi, A., Kapoor, A., Shayegan, B., Austin, R. C. & Al-Nedawi, K. 2018. Thrombotic characteristics of extracellular vesicles derived from prostate cancer cells. *The Prostate,* 78**,** 953-961.

Başeskioğlu, B., Duman, B. B., Yıldız, B., Çil, T., Dinçer, M. & Bayram, E. 2019. Pretreatment neutrophil-to-lymphocyte ratio and Mean Platelet Volume in castration-resistant prostate cancer patients treated with first-line docetaxel.

Börekoğlu, A., Deniz, A., Hüseyin, E., Altunkol, A., Karsli, O. & Yaylaci, S. 2020. Do Hematological Parameters Have a Significant Role in Predicting the Results of Prostate Biopsies?

Bowen, R. C., Little, N. A. B., Harmer, J. R., Ma, J., Mirabelli, L. G., Roller, K. D., Breivik, A. M., Signor, E., Miller, A. B. & Khong, H. T. 2017. Neutrophil-to-lymphocyte ratio as prognostic indicator in gastrointestinal cancers: a systematic review and meta-analysis. *Oncotarget,* 8**,** 32171.

Daniyal, M., Siddiqui, Z. A., Akram, M., Asif, H., Sultana, S. & Khan, A. 2014. Epidemiology, etiology, diagnosis and treatment of prostate cancer. *Asian Pacific Journal of Cancer Prevention,* 15**,** 9575-9578.

Davis, N. G. & Silberman, M. 2021. Bacterial acute prostatitis. *StatPearls [Internet]*. StatPearls Publishing.

De Jager, C. P., Van Wijk, P. T., Mathoera, R. B., De Jongh-Leuvenink, J., Van Der Poll, T. & Wever, P. C. 2010. Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Critical care,* 14**,** 1-8.

De Wit, R., Wülfing, C., Castellano, D., Kramer, G., Eymard, J.-C., Sternberg, C., Fizazi, K., Tombal, B., Bamias, A. & Carles, J. 2021. Baseline neutrophil-to-lymphocyte ratio as a predictive and prognostic biomarker in patients with metastatic castration-resistant prostate cancer treated with cabazitaxel versus abiraterone or enzalutamide in the CARD study. *ESMO open*, 6**,** 100241.

Elsayed, W. M. 2015. *Determination of Platelet Counts Platelet Indices and Coagulation Parameters among Sudanese Pregnant Women with Preeclampsia.* Alzaeim Alazhari University.

Faria, S. S., Fernandes Jr, P. C., Silva, M. J. B., Lima, V. C., Fontes, W., Freitas-Junior, R., Eterovic, A. K. & Forget, P. 2016. The neutrophil-to-lymphocyte ratio: a narrative review. *ecancermedicalscience,* 10.

Feng, J.-F., Yang, X., Chen, S., Zhao, Q. & Chen, Q.-X. 2016. Prognostic value of plasma D-dimer in patients with resectable esophageal squamous cell carcinoma in China. *Journal of Cancer,* 7**,** 1663.

Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D.M., Piñeros, M., Znaor, A. and Bray, F. (2019). Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International journal of cancer*, *144*(8), pp.1941-1953.

Fountain, J. H. & Lappin, S. L. 2021. Physiology, Platelet. *StatPearls [Internet]*. StatPearls Publishing.

Fu, S., Zhang, X., Niu, Y. & Wang, R.-T. 2018. Prostate specific antigen, mean platelet volume, and platelet distribution width in combination to discriminate prostate cancer from benign prostate hyperplasia. *Asian Pacific Journal of Cancer Prevention: APJCP*, 19**,** 699.

Fukuokaya, W., Kimura, T., Urabe, F., Kimura, S., Tashiro, K., Tsuzuki, S., Koike, Y., Sasaki, H., Miki, K. & Egawa, S. 2020. Reply to Cengiz Beyan and Esin Beyan. Mean platelet volume may not be a significant prognostic factor in patients with metastatic castration-resistant prostate cancer. Int J Clin Oncol 97 (2), 3–4, 2020. *International Journal of Clinical Oncology,* 25**,** 2177-2178.

Giacomini, A., Grillo, E., Rezzola, S., Ribatti, D., Rusnati, M., Ronca, R. & Presta, M. 2021. The FGF/FGFR system in the physiopathology of the prostate gland. *Physiol Rev,* 101**,** 569-610.

Goubran, H. A., Kotb, R. R., Stakiw, J., Emara, M. E. & Burnouf, T. 2014. Regulation of tumor growth and metastasis: the role of tumor microenvironment. *Cancer growth and metastasis,* 7**,** CGM. S11285.

Graziano, V., Grassadonia, A., Iezzi, L., Vici, P., Pizzuti, L., Barba, M., Quinzii, A., Camplese, A., Di Marino, P. & Peri, M. 2019. Combination of peripheral neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio is predictive of pathological complete response after neoadjuvant chemotherapy in breast cancer patients. *The Breast,* 44**,** 33-38.

Guo, J., Fang, J., Huang, X., Liu, Y., Yuan, Y., Zhang, X., Zou, C., Xiao, K. & Wang, J. 2018. Prognostic role of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in prostate cancer: a meta-analysis of results from multivariate analysis. *International Journal of Surgery,* 60**,** 216-223.

Hong, S. K., Ko, D. W., Park, J., Kim, I. S., Doo, S. H., Yoon, C. Y., Park, H., Lee, W. K., Kim, D. S. & Jeong, S. J. 2010. Alteration of antithrombin III and D-dimer levels in clinically localized prostate cancer. *Korean Journal of Urology,* 51**,** 25-29.

Huszno, J., Kołosza, Z., Mrochem-Kwarciak, J., Telka, E., Jochymek, B. & Miszczyk, L. 2022. Role of neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, lymphocyte-monocyte ratio and platelets in prognosis of patients with prostate cancer. *Oncology Letters,* 24**,** 1-6.

Ittmann, M. 2018. Anatomy and histology of the human and murine prostate. *Cold Spring Harbor perspectives in medicine,* 8**,** a030346.

Johnson, E. D., Schell, J. C. & Rodgers, G. M. 2019. The D-dimer assay. *American journal of hematology,* 94**,** 833-839.

Kalkan, S. & Caliskan, S. 2020. High D-dimer levels are associated with prostate cancer. *Revista da Associação Médica Brasileira,* 66**,** 649-653.

Kasap, Y., Uzun, E., Keseroglu, B. B., Tastemur, S., Ceylan, C. & Polat, M. E. 2021. Is There a Relationship Between Biochemical Recurrence and Insulin-like Growth Factor-1 in Patients with Surgical Border Negative Radical Prostatectomy? *Grand Journal of Urology,* 1.

Kawahara, T., Kato, M., Tabata, K., Kojima, I., Yamada, H., Kamihira, O., Tsumura, H., Iwamura, M., Uemura, H. & Miyoshi, Y. 2020. A high neutrophil-to-lymphocyte ratio is a poor prognostic factor for castration-resistant prostate cancer patients who undergo abiraterone acetate or enzalutamide treatment. *BMC cancer,* 20**,** 1-8.

Korniluk, A., Koper-Lenkiewicz, O. M., Kamińska, J., Kemona, H. & Dymicka-Piekarska, V. 2019. Mean platelet volume (MPV): new perspectives for an old marker in the course and prognosis of inflammatory conditions. *Mediators of inflammation,* 2019.

Kutlucan, L., Kutlucan, A., Basaran, B., Dagli, M., Basturk, A., Kozanhan, B., Gur, M., Senocak, E. & Kos, M. 2016. The predictive effect of initial complete blood count of intensive care unit patients on mortality, length of hospitalization, and nosocomial infections. *Eur Rev Med Pharmacol Sci,* 20**,** 1467-1473.

Lattanzi, S., Brigo, F., Trinka, E., Cagnetti, C., Di Napoli, M. & Silvestrini, M. 2019. Neutrophil-to-lymphocyte ratio in acute cerebral hemorrhage: a system review. *Translational stroke research,* 10**,** 137-145.

Lawrentschuk, N., Ptasznik, G. & Ong, S. 2021. Benign prostate disorders. *Endotext [Internet]*.

Lee, S., Huh, S. J., Oh, S. Y., Koh, M. S., Kim, S. H., Lee, J. H., Han, J. Y., Choi, H. J., Kim, S. J. & Kim, H. J. 2017. Clinical significance of coagulation factors in operable colorectal cancer. *Oncology Letters,* 13**,** 4669-4674.

Li, S., Silvestri, V., Leslie, G., Rebbeck, T. R., Neuhausen, S. L., Hopper, J. L., Nielsen, H. R., Lee, A., Yang, X. & Mcguffog, L. 2022. Cancer risks associated with BRCA1 and BRCA2 pathogenic variants. *Journal of Clinical Oncology,* 40**,** 1529.

Liu, Y.-L., Lu, J.-K., Yin, H.-P., Xia, P.-S., Qiu, D.-H., Liang, M.-Q., Qu, J.-F. & Chen, Y.-K. 2020. High neutrophil-to-lymphocyte ratio predicts hemorrhagic transformation in acute ischemic stroke patients treated with intravenous thrombolysis. *International Journal of Hypertension,* 2020.

Lokant, M. & Naz, R. 2015. Presence of PSA auto-antibodies in men with prostate abnormalities (prostate cancer/benign prostatic hyperplasia/prostatitis). *Andrologia,* 47**,** 328-332.

Lokeshwar, S. D., Harper, B. T., Webb, E., Jordan, A., Dykes, T. A., Neal Jr, D. E., Terris, M. K. & Klaassen, Z. 2019. Epidemiology and treatment modalities for the

management of benign prostatic hyperplasia. *Translational andrology and urology,* 8**,** 529.

Magri, V., Boltri, M., Cai, T., Colombo, R., Cuzzocrea, S., De Visschere, P., Giuberti, R., Granatieri, C. M., Latino, M. A. & Larganà, G. 2018. Multidisciplinary approach to prostatitis. *Archivio Italiano di Urologia e Andrologia*, 90**,** 227-248.

Maki, H. A. H. 2022. General Oncology Care in Sudan. *Cancer in the Arab World*. Springer, Singapore.

Matsumoto, M. & Yamamoto, S. 2021. AAUS guideline for acute bacterial prostatitis 2021. *Journal of Infection and Chemotherapy,* 27**,** 1277-1283.

Michalak, A., Cichoż-Lach, H., Guz, M., Kozicka, J., Cybulski, M. & Jeleniewicz, W. 2021. Plateletcrit and mean platelet volume in the evaluation of alcoholic liver cirrhosis and nonalcoholic fatty liver disease patients. *BioMed Research International,* 2021.

Murray, N. P., Fuentealba, C., Salazar, A. & Reyes, E. 2020. Platelet-to-lymphocyte ratio and systemic immune-inflammation index versus circulating prostate cells to predict significant prostate cancer at first biopsy. *Turkish Journal of Urology,* 46**,** 115.

Ng, K. L. 2021. The Etiology of Prostate Cancer. *Exon Publications***,** 17-27.

Nickavar, A. & Sadeghi-Bojd, S. 2020. Alteration of platelet indices in young children with acute pyelonephritis. *International Journal of Pediatrics,* 8**,** 11743-11749.

Palma Anselmo, M., Nobre De Jesus, G., Lopes, J. M., Victorino, R. M. & Meneses Santos, J. 2016. Massive bleeding as the first clinical manifestation of metastatic prostate cancer due to disseminated intravascular coagulation with enhanced fibrinolysis. *Case Reports in Hematology,* 2016.

Park, J.-S., Seo, K.-W., Choi, B.-J., Choi, S.-Y., Yoon, M.-H., Hwang, G.-S., Tahk, S.-J. & Shin, J.-H. 2018. Importance of prognostic value of neutrophil to lymphocyte ratio in patients with ST-elevation myocardial infarction. *Medicine,* 97.

Pernar, C. H., Ebot, E. M., Wilson, K. M. & Mucci, L. A. 2018. The epidemiology of prostate cancer. *Cold Spring Harbor perspectives in medicine,* 8**,** a030361.

Pirola, G. M., Verdacchi, T., Rosadi, S., Annino, F. & De Angelis, M. 2019. Chronic prostatitis: current treatment options. *Research and reports in urology,* 11**,** 165.

Pogorzelska, K., Krętowska, A., Krawczuk-Rybak, M. & Sawicka-Żukowska, M. 2020. Characteristics of platelet indices and their prognostic significance in selected medical condition–a systematic review. *Advances in Medical Sciences,* 65**,** 310-315.

Pontari, M. 2020. Definition and epidemiology of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS).

Posch, F., Riedl, J., Reitter, E. M., Crowther, M. J., Grilz, E., Quehenberger, P., Jilma, B., Pabinger, I. & Ay, C. 2020. Dynamic assessment of venous thromboembolism risk in patients with cancer by longitudinal D-Dimer analysis: A prospective study. *Journal of Thrombosis and Haemostasis,* 18**,** 1348-1356.

Rawla, P. 2019. Epidemiology of prostate cancer. *World journal of oncology,* 10**,** 63.

Rudzinski, J. K., Govindasamy, N. P., Lewis, J. D. & Jurasz, P. 2020. The role of the androgen receptor in prostate cancer-induced platelet aggregation and platelet-induced invasion. *Journal of Thrombosis and Haemostasis,* 18**,** 2976-2986.

Russell, M. R., Liu, Q., Lei, H., Kazlauskas, A. & Fatatis, A. 2010. The α-receptor for platelet-derived growth factor confers bone-metastatic potential to prostate cancer cells by ligand-and dimerization-independent mechanisms. *Cancer research,* 70**,** 4195-4203.

Saglam, H. S. & Cimen, H. I. 2021. D-dimer in the Diagnosis of Prostate Cancer. *Grand Journal of Urology*, 1.

Shah, R. B. & Zhou, M. 2019. Histologic Variants of Acinar Adenocarcinoma, Ductal Adenocarcinoma, Neuroendocrine Tumors, and Other Carcinomas. *Prostate Biopsy Interpretation.* Springer.

Sharma, D., Brummel-Ziedins, K. E., Bouchard, B. A. & Holmes, C. E. 2014. Platelets in tumor progression: a host factor that offers multiple potential targets in the treatment of cancer. *Journal of cellular physiology*, 229**,** 1005-1015.

Shen, Y., Wang, H., Chen, X., Li, W. & Chen, J. 2019. Prognostic significance of lymphocyte-to-monocyte ratio and platelet-to-lymphocyte ratio in patients with hepatocellular carcinoma undergoing transcatheter arterial chemoembolization and radiofrequency ablation. *OncoTargets and therapy,* 12**,** 7129.

Shimada, H., Takiguchi, N., Kainuma, O., Soda, H., Ikeda, A., Cho, A., Miyazaki, A., Gunji, H., Yamamoto, H. & Nagata, M. 2010. High preoperative neutrophil-lymphocyte ratio predicts poor survival in patients with gastric cancer. *Gastric cancer,* 13**,** 170-176.

Soliman, W. M., Sherif, N. M., Ghanima, I. M. & El-Badawy, M. A. 2020. Neutrophil to lymphocyte and platelet to lymphocyte ratios in systemic lupus erythematosus: relation with disease activity and lupus nephritis. *Reumatología Clínica,* 16**,** 255-261.

Song, W., Ding, N., Zhang, X., Liu, J., Wang, Y., Yan, J. & Liu, S. 2022. Mean Platelet Volume Enhances the Diagnostic Specificity of PSA for Prostate Cancer. *Frontiers in Surgery,* 9.

Strassel, C., Gachet, C. & Lanza, F. 2018. On the way to in vitro platelet production. *Frontiers in medicine,* 5**,** 239.

Sun, Y.-H., Cui, L., Chen, J., Wang, M., Liu, J.-J., Liu, X.-X. & Huang, X.-E. 2015. Analysis of relationships between prethrombotic states and cervical cancer. *Asian Pacific Journal of Cancer Prevention,* 16**,** 6163-6166.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A. & Bray, F. 2021. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians,* 71**,** 209-249.

Tarabrin, O., Suslov, O., Sukhonos, R., Volodychev, D., Basenko, I., Bugaenko, E. & Tarabrin, P. 2019. Correlation between fibrinogen and D-dimer levels with low-frequency piezoelectric thromboelastography (LPTEG) data in patients with confirmed prostate cancer.

Undas, A. 2020. Altered fibrin clot properties and fibrinolysis in patients with atrial fibrillation: practical implications. *EP Europace,* 22**,** 185-194.

Wada, Y., Uchiba, M., Kawano, Y., Kai, N., Takahashi, W., Honda, J., Tanoue, K.-I., Maeda, Y., Murakami, Y. & Eto, M. 2012. Severe bleeding tendency caused by a rare complication of excessive fibrinolysis with disseminated intravascular coagulation in a 51-year-old Japanese man with prostate cancer: a case report. *Journal of Medical Case Reports,* 6**,** 1-5.

Wang, F.-M. & Xing, N.-Z. 2021. Systemic Coagulation Markers Especially Fibrinogen Are Closely Associated with the Aggressiveness of Prostate Cancer in Patients Who Underwent Transrectal Ultrasound-Guided Prostate Biopsy. *Disease Markers,* 2021.

Wang, Z., Peng, S., Xie, H., Guo, L., Jiang, N., Shang, Z. & Niu, Y. 2018. Neutrophil–lymphocyte ratio is a predictor of prognosis in patients with castration-resistant prostate cancer: a meta-analysis. *Cancer Management and Research,* 10**,** 3599.

Watts, E. L., Perez-Cornago, A., Kothari, J., Allen, N. E., Travis, R. C. & Key, T. J. 2020. Hematologic Markers and Prostate Cancer Risk: A Prospective Analysis in UK BiobankHematologic Markers and Prostate Cancer Risk in UK Biobank. *Cancer Epidemiology, Biomarkers & Prevention,* 29**,** 1615-1626.

Wen, J., Yang, Y., Ye, F., Huang, X., Li, S., Wang, Q. & Xie, X. 2015. The preoperative plasma fibrinogen level is an independent prognostic factor for overall survival of breast cancer patients who underwent surgical treatment. *The Breast,* 24**,** 745-750.

Wu, L., Zou, S., Wang, C., Tan, X. & Yu, M. 2019a. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio in Chinese Han population from Chaoshan region in South China. *BMC cardiovascular disorders,* 19**,** 1-5.

Wu, Y.-Y., Zhang, X., Qin, Y.-Y., Qin, J.-Q. & Lin, F.-Q. 2019b. Mean platelet volume/platelet count ratio in colorectal cancer: a retrospective clinical study. *BMC cancer,* 19**,** 1-7.

Xu, Z., Zhang, J., Zhong, Y., Mai, Y., Huang, D., Wei, W., Huang, J., Zhao, P., Lin, F. & Jin, J. 2021. Predictive value of the monocyte-to-lymphocyte ratio in the diagnosis of prostate cancer. *Medicine,* 100.

Yin, J.-B., Li, N., Cui, M.-M., Wang, X. & Wang, R.-T. 2020. Reduced mean platelet volume levels predict shorter survival in patients with resectable pancreatic ductal adenocarcinoma and type 2 diabetes. *BMC gastroenterology,* 20**,** 1-7.

Yin, X., Xiao, Y., Li, F., Qi, S., Yin, Z. & Gao, J. 2016. Prognostic role of neutrophil-to-lymphocyte ratio in prostate cancer: a systematic review and meta-analysis. *Medicine,* 95.

Young, B., Woodford, P. and O'Dowd, G. (2013). *Wheater's functional histology E-Book: a text and color atlas*. Elsevier Health Sciences.

Zhang, J.-Y., Ge, P., Zhang, P.-Y., Zhao, M. & Ren, L. 2019. Role of Neutrophil to Lymphocyte Ratio or Platelet to Lymphocyte Ratio in Prediction of Bone Metastasis of Prostate Cancer. *Clinical Laboratory,* 65.

Zhang, S., Cui, Y.-L., Diao, M.-Y., Chen, D.-C. & Lin, Z.-F. 2015. Use of platelet indices for determining illness severity and predicting prognosis in critically ill patients. *Chinese medical journal,* 128**,** 2012-2018.

Zheng, C.-F., Liu, W.-Y., Zeng, F.-F., Zheng, M.-H., Shi, H.-Y., Zhou, Y. & Pan, J.-Y. 2017. Prognostic value of platelet-to-lymphocyte ratios among critically ill patients with acute kidney injury. *Critical care,* 21**,** 1-11.

Appendix

Appendix I

Sudan University of Science and Technology

College of Graduate Studies

Assessment of D-dimer, platelets count and platelets indices among Sudanese Men with prostate cancer in Khartoum state – 2022

Questionnaire No ( )

Basic information

Age : …… Residence : ……

Mobile number ……... Diagnosis : …….

Duration : …… Metastasis : ……

History of

Bleeding ( ) Stroke ( ) DVT ( )

Drugs history

Aspirin ( ) chemotherapy ( ) others ( )

If yes : currently ( ) on the past ( ) duration ( )

History of other chronic disease : ( ) if yes Type : ……..

Laboratory investigation results

1. **Hematological parameters**

Platelets count : ……cell/L Neutrophil …….. %

MPV : …….FL Neutrophil …….. cell/L

PDW : ……..FL Lymphocyte …… %

P-LCR : ……% Lymphocyte …….. cell/L

PCT : ……% PLR ……

2. **D-dimer ……** NLR ……..

Appendix II

جامعة السودان للعلوم و التكنولوجيا

كلية الدراسات العليا

كلية علوم المختبرات الطبية

مشروع لقياس مستوى الدي دايمرو حساب الصفائح الدموية و مؤشرات الصفائح الدموية، نسبة العدلات للمفاويات و نسبة الصفائح الدموية لليمفاويات بين الرجال السودانيين المصابين بسرطان البروستاتا بولاية الخرطوم

استمارة الموافقة على بحث لقياس مستوى الدي دايمرو حساب الصفائح الدموية و مؤشرات الصفائح الدموية، نسبة العدلات للمفاويات و نسبة الصفائح الدموية لليمفاويات بين الرجال السودانيين المصابين بسرطان البروستاتا بولاية الخرطوم

يعتبر السرطان احد الأمراض التي تسبب حالة فرط التخثر التي تؤدي الى تكون الجلطات التي يمكن أن تؤدي للوفاة.

أنا باحثة من كلية علوم المختبرات الطبية بجامعة السودان أقوم ببحث لقياس مستوى الدي دايمر و حساب الصفائح الدموية و مؤشرات الصفائح لمعرفة ما اذا كان المرضى المصابين بسرطان البروستاتا عرضة للجلطات أم لا حتى يتسنى عمل الفحوصات اللازمة و توفير العلاج لهم.

و حتى أستطيع أن أقوم بهذا أطلب منك المشاركة في هذا البحث. و اذا وافقت سأقوم بأخذ عينة دم لإجراء الفحوصات.

و أخذ هذه العينة لن يعرضك للخطر و سأستخدم أدوات معقمة لأخذ العينات.

و أعلم بأن عدم موافقتك على المشاركة لن يحرمك من حقك في العلاج و الرعاية الصحية المطلوبة.

و أنك لن تتلقى أي عائد مادي لمشاركتك في البحث و ستكون مشاركتك طوعية.

و أود اخطارك بأن المعلومات الخاصة بك ستكون سرية و لن يطلع عليها الآخرون ما عدى

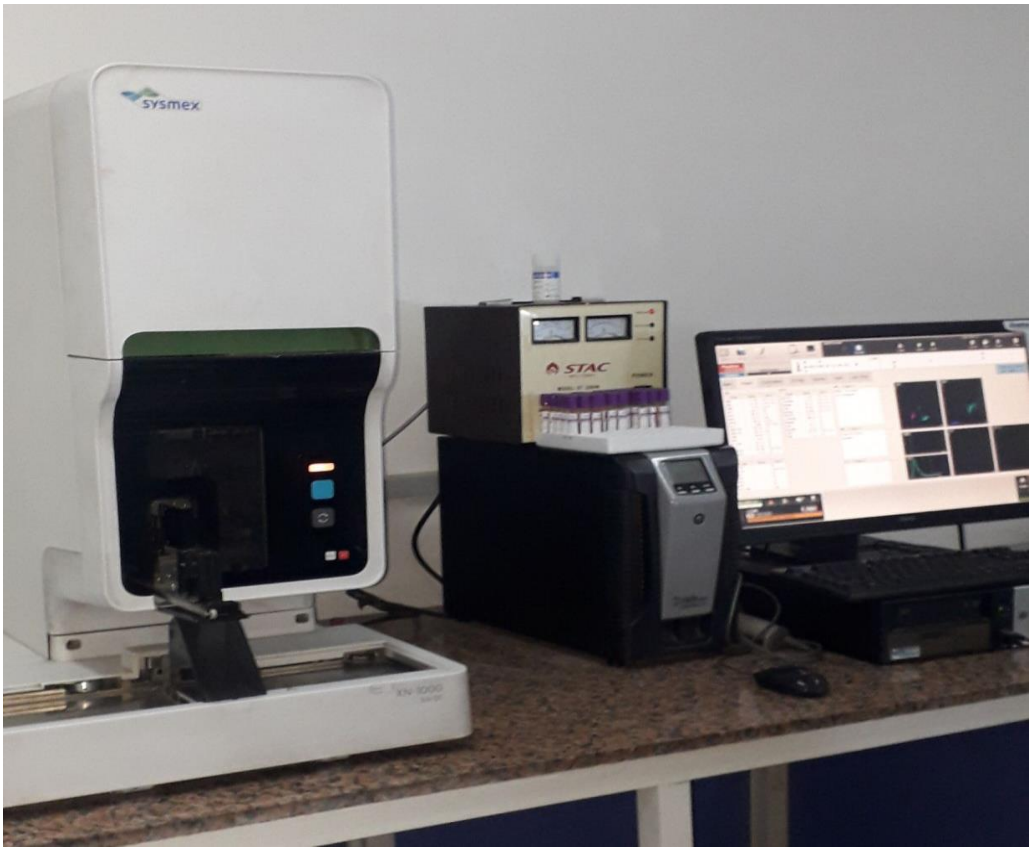
أود موافقتك على المشاركة في هذا البحث و يمكنك أن تسأل و تستفسر عن البحث و مشاركتك فيه قبل الموافقة النهائية. و أرجو اثبات موافقتك بإمضاء الاستمارة بعد قارئتها جيدا

أنا ..... أوكد فهمي لمحتوى الاستمارة و أفيد بموافقتي على المشاركة في البحث و أعلم أنه من حقي رفض المشاركة و الانسحاب في أي وقت و لن يؤثر ذلك على حقوقي.

و أوافق على أخذ العينة للبحث.

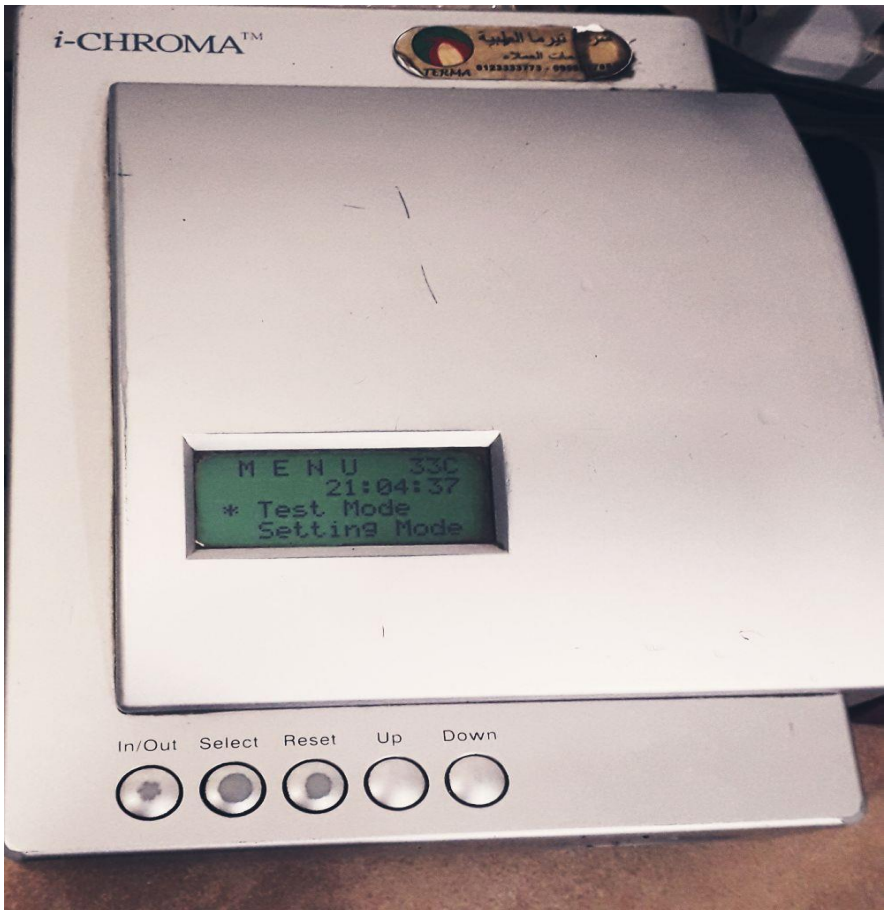
الامضاء : .....

Appendix III



Sysmex XN hematology analyzer

Appendix IV



I-chroma ™