# Chapter One

## Introduction and Literature Review

### 1.1 Introduction

Carcinoma of the prostate is the most common visceral cancer in male, ranking as the second most common cause of cancer related death in men older than 50 years of age, after carcinoma of the lung it is predominantly a disease of older males, with a peak incidence between the ages of 65 and 75 years , latent cancers of the prostate are even more common than those that are clinically apparent, with an overall frequency of more than50% in men older than 80 years of age (Vinay *et a.*,2003).

Cancer is a risk factor for thromboembolic disease, and patients with cancer are estimated to be around four times more likely to develop a thrombosis than a similar individual without cancer. Treatments for cancer might also increase the risk of thromboembolic disease. For prostate cancer, deep-venous thrombosis (DVT) and thromboembolism are common complications the underlying mechanisms for the higher risk of thromboembolic disease could have several explanations. First, a baseline risk might be present because of physiological alterations due to the tumor, which seems to be supported by the fact that the risk of thromboembolic disease increases as tumor stage increases. Second, the different patterns of risk associated with different types of treatment. **(**Reinhold *et al*.,2007)

Approximately 11% of the Caucasian population is homozygous for this MTHFR mutation, with a threefold increased risk of thrombosis (Amer and Amitava,2015).

MTHFR C677T (and A1298C) polymorphism are most common gene associated with increase cancer risk (gastric,breast,prostate and testicular cancer)(David,2018).

Methylene tetra hydrofolate reductase MTHFR is a critical part of methylation pathway which are involve transfer of methyl groups to amino acid, proteins, enzymes and DNA in every cell and every tissue in the human body. This is a crucial metabolic process required for living (David,2018).

## 1.2 Literature Review

### 1.2.1 Thrombophilia (hypercoagulability)

Thrombophilia is defined as an abnormality of the coagulation or fibrinolytic system that results in a hypercoagulable state and increases the risk of an individual for a thrombotic event in which intravascular thrombus formation may be arterial or venous (Amer and Amitava, 2015).

Thrombi are solid masses or plug formed in the circulation from blood constituent .platelets and fibrin form the basic structure. Their clinical significance results from ischemia from local vascular obstruction or distant embolization. Thrombi are involved in the pathogenesis of myocardial infraction, cerebrovascular disease, peripheral arterial disease and deep vein occlusion(Hoff brand *et al*., 2006).

Venous thrombosis has an overall incidence of less than 1 per 1000 people, and in the pediatric population it is approximately 1in 100,000. (Amer *et al*., 2015).

#### 1.2.1.1 The risk factor for thrombophilia

##### 1.2.1.1.1 Heritable thrombophilia:

Inherited tendency for venous thrombosis. Familial thrombophilia is likely a multiple gene disorder, and family members may have one or more identifiable defects probably in combination with additional unknown defects.

The heritable thrombophilia shown to be associated with at least a twofold increased risk of venous thrombosis (Amer and Amitava, 2015).

Most inherited hypercoaguable states associated with increased risk of venous thromboembolism are deficiencies of one or more anticoagulant protein or defect in coagulation factors that increase their level of expression or make them no longer subject to inhibition or regulation by anticoagulant protein they may also be metabolic problems such as homocysteinemia where toxic levels of homocysteine be toxic to the endothelium and promote thrombosis (Griffin and Neal ,2013)

Inherited thrombophilia should be used for individuals with predisposing genetic defect and usually suspected in patient with one or more following clinical features:

Idiopathic thrombosis Thrombosis at young age Recurrent Thrombosis
Thrombosis at unusual site (e.g. axillary, sagittal sinus)(Reinhold *et al*, 2007)

- **Inherited hypercoagulable states include**

Activated protein C resistance due to factor V Leiden mutation ,prothrombin gene Mutation, antithrombin deficiency, protein C deficiency,protein S deficiency,elevated factorVIII level ,dysfibrinogenemia(rare) and hyperhomocysteinemia (Reinhold *et al*, 2007).

**1.2.1.1.2 Acquired thrombophilia:**

These may cause thrombosis in patients with out another identifiable abnormality but are most likely to do so if an inherited predisposing abnormality is also present.More common than inherited cause (Reinhold *et al*.,2007 )

- **Acquired hypercoagulable states include:**

Antiphospholipid syndrome (Lupus anticoagulant), Malignancy ,Nephrotic syndrome ,postoperative state, immolilization, old age, pregnancy and oral contraceptives or other estrogen use (Reinhold *et al* .,2007)

#### 1.2.1.2 Investigation of thrombophilia

Investigation of thrombophilia may include Blood count and erythrocyte sedimentation rate(ESR), Blood film examination, Prothrombin time (PT) and APTT, Anticardiolipin and anti B2-GPI antibodies, Thrombin time and reptilase time, Fibrinogen assay, Activated protein C (APC)resistance test and DNA analysis for factor V leiden, Antithrombin –immunological and functional assay, Protein C and protein S-immunological and functional assay, Prothrombin gene analysis for the G20210A variant, Plasma homocystein estimation and Test for CD59 and CD55 expression (PNH) red cells if paroxysmal nocturnal haemoglobinuria is suspected (Hoff brand *et al* 2006).

#### 1.2.1.3 Management of thrombophilia

Decisions about medical intervention due to the presence of one or more genetic defects should be based on careful consideration of the clinical picture, including the patient's family history. The risk of bleeding complications due to anticoagulant therapy must always be weighed against the benefits of the anticoagulation effe ct, especially if an oral anticoagulant is used for periods exceeding 3–6 months, after which the risk of thrombotic recurrence probably declines. When the FV Leiden allele is present in homozygous form, or when heterozygosity is combined with second genetic defect,prophylactic treatment with heparin or oral anticoagulants is recommended in situations known to be associated with a high risk of

thromboembolic complications, such as surgery or pregnancy, even if the patient has never experiencedany thrombosis or has no family history of such complications. For heterozygous, asymptomatic carriers lacking a family history of thrombosis, short-term prophylaxis has been recommended in high-risk situations. Treatment of symptomatic heterozygous patients should be initiated as for any other patient with thrombotic events. Patients with combined defects and probably also patients with single gene defects may be at increased risk of recurrence and should accordingly be given extended anticoagulation therapy beyond 6 months, even after an isolated thromboembolic event(drew and john,2005).

#### 1.2.1.4 Hyperhomocysteneimia

Homocysteine is amino acid that does not appear in protein but is an intermediate in the metabolism of methionine a sulfur containing essential amino acid. Reaction of the metabolic pathway in which homocysteine participate require folic acid, cyanocobalamine (vitB12), orpyridoxine (vitB6) as cofactor, deficiency of these vitamins may cause accumulation of homocysteine to high level in the blood steam (acquired hyperhomocysteneimia) (Griffin and Neal, 2013).

Inherited cause of hyperhomocystenimia include deficiencies of 5,10-methylenetetrahydrofolatereductase (MTHFR) or cystathionine-β-synthase enzymes due to defective genes that encode such enzymes ( Amer and Amitava ,2015).

Hyperhomocysteinemia(inherited or acquired), it is a risk factor for cardiovascular diseases as well as thrombophilia. Because homocysteine may be toxic to endothelial cells and accelerate atherosclerosisInherited causes include deficiencies of 5, 10-methylenetetrahydrofolatereductase (MTHFR) or cystathionine-β-synthase

enzymesdue to defective genes that encode such enzymes (Amer and Amitava ,2015) (Griffin and Neal, 2013).

5,10-methylenetetrahydrofolatereductase(MTHFR) enzyme is an enzyme infolate-dependent homocysteineremethylation, catalyzing the reduction of5,10-methylenetetrahydrofolate(substrate) into 5-methyltetrahydrofolate(product).(Amer,Amitava 2015)

### 1.2.1.5 MTHFR C677T polymorphism influence thrombosis

MTHFR is composed of an N-terminal catalytic domain and a C-terminal regulatory domain. MTHFR has at least two promoters and two isoforms Single base pair substitution at nucleotide location 677 (thymine replaces cytosine:677 C.T) in the MTHFR gene results in substitution of alanine by valine atlocation 223 (A223V) in the 5,10-methylenetetrahydrofolate reductaseenzyme with decreased activity causing hyperhomocystinemia. As a result, these individuals are at higher thrombotic risk (mechanisms: blood vessel injury, coagulation activation, fibrinolysis inhibition, and platelet activation) (Amer and Amitava, 2015).

Approximately 11% of the Caucasian population is homozygous for this mutation, with a threefold increased risk of thrombosis. Although this is the most common mutation, at least 40 different mutations of the MTHFR gene have been described in individuals with homocystinuria (Amer and Amitava, 2015).

### 1.2.1.6 Deep Venous thrombosis and pulmonary embolism

DVT of the extremity is often the precursor of PE, though both may become symptomatic at the same time, and it is possible that PE may occur without

symptomatic DVT, due to complete embolism of nascent thrombus before complete occlusion of the vein occurs.

The post thrombotic syndrome (PTS) is an important complication of DVT. It is caused by venous hypertension from outflow obstruction and valvular injury and varies from mild edema with little discomfort to incapacitating limb swelling with pain and ulceration(Griffin and Neal, 2013).

**1.2.1.7 Signs and symptoms of DVT and PE (**Griffin and neal,2013 **)**

| **Sign/symptoms** | **Occurrence%** |
|---|---|
| **Deep vein thrombosis** | |
| Calf or leg swelling | 88% |
| Pain | 56% |
| Tenderness | 55% |
| Erythema | 34% |
| Palpable cord | 6% |
| **Pulmonary embolism** | |
| Dyspnea | 77% |
| Tachypnea | 70% |
| Tachycardia | 43% |

| | |
|---|---|
| Hypoxia/cyanosis | 18% |
| Hemoptysis | 13% |
| Syncope | 10% |
| hypotension | 10% |

### 1.2.1.8 Venous thromboembolism and cancer:

The association between cancer and venous thromboembolism is well established. Thrombotic event rank second only to the direct effects of cancer as cause of death in cancer patients.(Avictor *et al*., 2016).VTE frequently complicates malignancy and results in significant morbidity and mortality. The estimated prevalence of VTE in patients with cancer is 10% to 15%; sites have been associated with VTE associated with malignancy: intra-abdominal and bilateral lower extremity DVT. As long as the cancer is active, the increased risk for VTE is present (Griffin and Neal ,2013).

Some pathogenic factors for thrombosis in cancer patients:

Stasis (Immobility / surgery), treatment (Surgery, chemotherapy, radiotherapy, and adjuvant therapy), Vessel wall/ endothelial perturbation (local tumor infiltration , central venous catheters) and hypercoagulability (infection, cytokine related prothrombotic change, tissue factor and cancer procoagulant expression on tumour cell which may result from proto-oncogen expression and tumour suppressor gene inhibition, disseminated intravascular coagulation,increased platelet activation,

prothrombatic properties of mucins secreted by adenocarcinoma and altered fibrinolysis).

The development of venous thromboembolism in the context of malignancy is generally a poor prognostic feature often indicative of advanced and aggressive malignancy(Avictor *et al*., 2016).

### 1.2.1.9 Prophylaxis and treatment of venous thromboembolism in cancer patients

Low-molecular-weight heparin (LMWH) is more effective than oral anticoagulants in reducing the risk of recurrent VTE without increasing the risk of bleeding in patients withcancer and acute VTE. Products such as dalteparin, enoxaparin, nadroparin, and tinzaparin as well as the synthetic factor Xainhibitor, fondaparinux are approved by the Food and Drug Administration (FDA) for the prophylaxis and treatment of VTE. Ingeneral, unfractionated heparin (UFH), LMWH, fondaparinux, and oral anticoagulants are the mainstay of therapy. SinceLMWH undergoes renal excretion, patients with kid ney impairment who receive LMWH should be monitored by measurementof the anti-factor Xa activity. The creatinine clearance should be estimated (or calculated) before initiation of LMWH in elderly patients, since they may have renal dysfunction despite having normal creatinine values (Avictor *et al*., 2016).

### 1.2.1.10 MTHFR and methylation

The MTHFR gene is involved in the metabolism of both folate and one –carbon.

One carbon metabolism is required for DNA synthesis and the conversion of homocysteine in to S-adenosyle methionine or SAM . SAM is critical methyl donating component required for successful DNA,RNA,and protein methylation.

The folate metabolizing enzyme known as5,10 methylene tetrahydrofolate reductase also plays a role in regulating DNA synthesis and production of SAM.

The following functions are reliant on adequate methylation signaling Detoxification, histamine tolerance, stress management, DNA and RNA protection and repair, and neurotransmitter myelination (nerve protection) (David,2018).

### 1.2.1.11 MTHFR C677T polymorphism and cancer

There are major disruption of MTHFR that can impact the risk of cancer developing ,of the many biological pathways it is involved with the conversion of homocysteine into methionine can be disrupted when the concentration of precursor molecules exceed normal . for instance , instead of thiamine , uracil may be incorporated at exceedingly high numbers . this repetitive genetic variance increases the risk for DNA damage and further genetic mutation .

As previously mentioned SAM is necessary for the process of methylation in DNA .when this methionine compound becomes altered in anyway , DNA and genetic expression can be modified . for example . when MTHFR decreases in activity a phenomenon called hypo methylation occurs (from low SAM levels ). Without an adequate supplier of methyl group , this loss burdens the body with the need to prioritize its need for methyl groups based on immediate stressors .

Despite being first recognized in 1983, the role of hypo methylation – in which the need for methyl groups is far greater than what is available has not been fully addressed until the 21$^{st}$ century. Hypo methylation has since shown to play adirect role in carcinogenic tissue growth and is significantly linked to cancer metastasis.to date ,hypo methylation is found in all cancer types ,increase with the progression of a tumor and causes a variety of cancerous activity indifferent individuals. (David,2018) .

### 1.2.2 Prostate gland

Prostate gland compact encapsulated organ that weight about 20 gram and shaped like walnut, measuring approximately 2cm (anteroposterior diameter) by 3cm (vertical diameter) by 4cm(transverse diameter at the base

The prostate gland secrete slightly milky fluid that weakly acidic and rich in citric acid,seminalplasmin and prostate-specific antigen (PSA). The citric acid is a nutrient for sperm health , the seminalplasmin is an antibiotic that combats urinary tract infections in the male , and PSA act as an enzyme to help liquefy semen following ejaculation.(note the slightly acidic secretion of the prostate doesn't cause seminal fluid still functions to neutralize the acidity of the vagina)(Mckinley *et al*.,2006).

The size and activity of prostate are under the influence of sex hormones such as testosterone (hormone produced mainly by testicle), and it's metabolitedihydrotestosterone (DHT ) , the prostate becomes functionally active from the age of puberty(Neeta and Kulkarni, 2014).

#### 1.2.2.1 Location

The prostate lies between the urinary bladder and urogenital diaphragm. It lies behind the lower margin of pubic symphsis and infront of rectal ampulla. It surrounds the initial 3 cm of the urethra.( Neeta V Kulkarni 2014)

#### 1.2.2.2 Structure

The prostate consist of stroma and glandular tissue the Fibro muscular stroma( one third of prostate) composed of smooth muscle and fibrous tissue . the smooth muscle is continuous above with smooth muscle of bladder wall. A peculiar feature

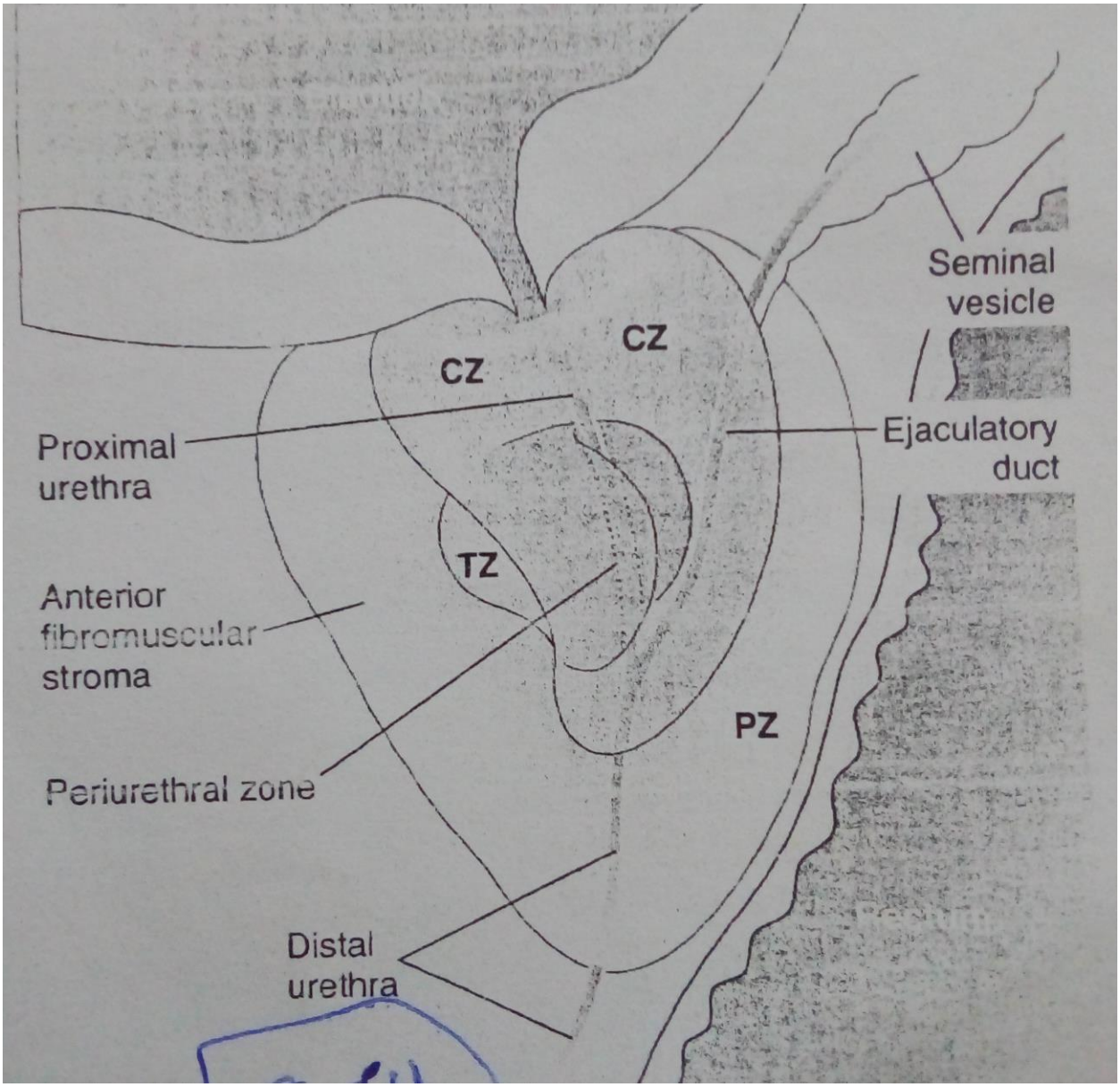
of the muscle in the prostate is the presence of striated muscle subjacent to the true capsule. The function the intra glandular smooth muscle is to compress the follicles to facilitate their drainage in prostatic urethra. The function of striated muscle is probably to expand the prostatic urethra to accommodate seminal fluid.

The glandular tissue make up (approximately two third of prostate) is arranged in three concentric zone. The peripheral zone consists of long branching gland, whose ducts curve to rich the posterior wall of the prostatic sinuses below the level of colliculusseminalis. The internal zone consists of sub mucosal glands, whose ducts open on the floor of the prostatic sinuses at the level of colliculusseminalis .The sub mucosal glands are prone to benign hypertrophy of prostate (BHP). The inner most zone consists of simple mucosal glands surrounding the upper part of prostatic urethra( Neeta and Kulkarni, 2014).

### 1.2.2.3 Surgical lobes

The prostate is incompletely divided in to five lobes, anterior lobe or isthmus lies in front of urethra. This lobe is the connecting bridge between the anterior ends of the two lateral lobes .it is devoid of glandular tissue hence adenoma never occurs in this lobe, posterior lobe is situated behind the urethra and below the ejaculatory duct .it connects the posterior ends of the two lateral lobe .it contains glandular tissue and is the site of primary carcinoma . this lobe palpable per rectum, median lobe lies between urethra and ejaculatory ducts .it is a wedge shaped lobe in contact with trigone of bladder.it contains more glandular tissue than other lobes and is more susceptible to benign hypertrophy of prostate(BHP). The median lobe is not palpable per rectum and right lateral lobe on the right side of urethra and the left lateral lobe on its left side contain numerousglands,which may give rise to adenoma( Neeta and Kulkarni ,2014).

Figure 1.1Prostate gland (Vinay,*et al*.2002)



### 1.2.2.4 Histological structure

The prostate is an exocrine compound tubule-alveolar gland,it surrounds the first part of the male urethera,it formed of a stroma of C.T and a parenchyma of secretory acini.

The C.T stroma of the prostate is formed of capsule, trabeculae and reticular C.T,The capsule is thin and fibro-muscular,formed of C.T.rich in smooth muscles

The trabeculae are thin fibro-muscular septa that extend from the capsule towards the prostatic urethra dividing the gland in to lobules,the reticular C.T it is formed of A fine network of reticular cells and reticular fibers.

The parenchymatous tissue of the prostate is made of a large number of individual glands; these glands open by separate ducts in to prostatic urethra.

The ejaculatory ducts subdivide the prostate in to three lobes each lobe is subdivided in to lobules that are made of compound tubule-alveolar secretory units.

These compound tubule-alveolar prostatic glands are distributed in three different concentric regions around the Vshaped male prostatic urethra. These three regions of the prostatic acini are distributed as follow:

Mucosal prostatic glands: they are located in the periurethral tissue they are the smallest type they may enlarge in old age to form prostatic adenoma.
The submucosal prostatic gland: they are arranged in a ring layer of connective tissue rich in smooth muscle, they surround the mucosal gland.
The main prostatic gland: they are situated in the outer and largest portion of the prostate. Their ducts open in to the posterior margins of the urethral sinuses

The epithelial lining of the secretory units of the prostate glands is tall columnar cells alternating with small flat or rounded cells in between the columnar cells.

In old men the prostatic secretion may contain calcified small bodies known as prostatic calcified concretions.(zakaria,1981)

### 1.2.2.5 Clinical significance

The most important categories of prostatic disease are inflammatory lesion (prostatitis), nodular hyperplasia, and carcinoma(Vinay *et al*., 2000).

#### 1.2.2.5.1Prostatitis

Inflammation of prostate may be acute or chronic.

#### 1.2.2.5.1.1Acute bacterial prostatitis

Is caused by same organism associated with other acute urinary tract infection particularly Escherichia coli and other gram negative rods. Mostpatients with acute prostatitis have concomitant infection of the urethra and urinary bladder in this case organism may rich the prostate by direct extension from the urethra or urinary bladder or by vascular channels from more distant sites(Vinay *et al* .,2000).

#### 1.2.2.5.1.2Chronic prostatitis

May follow obvious episode of acute prostatitis or it may develop insidiously without previous episodes of acute infection. In some case of chronic prostatitis bacteria similar to those responsible for acute bacterial prostatitis can be isolated (chronic bacterial prostatitis) .in other instance the presence of an increased number of leukocyte in the prostatic secretion attests to prostatic inflammation but

bacteriologic finding are negative ( chronic a-bacterial prostatitis or prostatodynia), account for cases of chronic prostatitis (Vinay *et al*., 2000)

**1.2.2.5.2 Benign prostatic hyper plasia (BPH)**

Non-cancerous enlargement of the prostate gland.BPH is a common disorder in older men; in fact , it is incidence is greater than 90% for men over 80 years of age. Hormonal changes in aging males are the cause of the excessive growth. (Mckinley *et al*., 2006).

In BPH, large discrete nodules form with in the prostate and compress the prostatic urethra. Thus the patient has,Hesitancy (difficult starting and stopping a stream of urine ), nocturia (urinating at night), Polyuria (more- frequent urination) and dysuria (painful urination) (Mckinley *et al*.,2006).

**1.2.2.5.3 Carcinoma of the prostate:-**

Carcinoma of the prostate is the most common visceral cancer in male, ranking as the second most common cause of cancer related death in men older than 50 years of age, after carcinoma of the lung it is predominantly a disease of older males, with a peak incidence between the ages of 65 and 75 years , latent cancers of the prostate are even more common than those that are clinically apparent, with an overall frequency of more than50% in men older than 80 years of age (Vinay *et al* .,2000).

**1.2.2.5.3.1 Morphology**

70-80% of prostate carcinoma arise in the outer (peripheral) glands and hence may be palpable as irregular hard nodules by D RE. Because of the peripheral location prostate cancer is less likely to cause urethral obstruction in it is initial stage than is nodular hyperplasia. Early lesions typically appear as ill , defined masses just

beneath the capsule . Metastases to regional pelvic lymph nodes may occur early. Locally advanced cancer often in filtered the seminal vesicles and periurethral zones of the prostate and may invade the adjacent soft tissue and the wall of urinary bladder. Invasion of the rectum is less common than is invasion of other contiguous structure because the rectum it separated from the lower genitourinary structure by connective tissue layer (nonvilliers fascia) (Vinay *et al*., 2000).

Microscopically most prostatic carcinoma are adenocarcinoma. The gland in carcinoma are not encircled by collagen or stromal cell the neoplastic glands are lined by a single layer of cuboidal cells with conspicuous nuclide basal (basal cell seen in normal or hyperplastic gland)(Vinay *et al*., 2000).

**1.2.2.5.3.2Clinical features**

Carcinoma of the prostate are often silent particularly during their early stage more extensive disease may produce signs and symptoms ( prostatism) including local discomfort and epidence of lower urinary tract obstruction similar in BPH . More aggressive carcinomas on the prostate are presents of metastases (Vinay *et al*., 2000). this metastases maybe:lymphatic initially perscral , iliac and par aortic lymph node, Retrograde via venous to lumber spine, blood stream causing wide spread metastases with marked predilection for the skeleton .bony metastases due to prostatic carcinoma are typically osteo sclerotic (new bone formation ).

(Roderick et al., 1992).

**1.2.2.5.3.3Epidemiology**

Race/ethnicity prostate cancer is more common and occurs at an earlier age in American blacks than in white, asins , or Hispanics, there is very low incidence in Chinese and Japanese .

Geography Prostate cancer is most common in North America, northwestern Europe, Australia, and on Caribbean islands. It is less common in Asia, Africa,

Central America, and South America.The reasons for this are not clear (Akaza,2009).

#### 1.2.2.5.3.4 Causes

Although the cause of carcinoma of the prostate remains unknown, clinical and experimental observation suggest that hormonal, genetic, and environmental factors may all play a role in its pathogenesis.

**1.2.2.5.3.4.1Hormonal factor**

cancer of prostate does not develop in males castrated before puberty, indicating that androgen likely play apart in its development.

**1.2.2.5.3.4.2Genetic factor** Much effort is focused in finding prostate cancer genes.gene's changes can either be inherited gene mutations Some gene mutations can be passed from generation to generation and are found in all cells in the body. These mutations are inherited. Inherited gene changes cause about 5% to 10% of prostate cancers. Cancer caused by inherited genes is called hereditary cancer. Several inherited mutated genes have been linked to hereditary prostate cancer, including *RNASEL* (formerly *HPC1*)The normal function of this tumor suppressor gene is to help cells die when something goes wrong inside them. Inherited mutations in this gene might let abnormal cells live longer than they should, which can lead to an increased risk of prostate cancer.

*BRCA1 and BRCA2* These tumor suppressor genes normally help repair mistakes in a cell's DNA (or cause the cell to die if the mistake can't be fixed). Inherited mutations in these genes more commonly cause breast and ovarian cancer in women. But changes in these genes (especially *BRCA2*) also account for a small number of prostate cancers.

DNA mismatch repair genes (such as *MSH2* and *MLH1*)These genes normally help fix mistakes (mismatches) in DNA that are made when a cell is preparing to divide into 2 new cells. (Cells must make a new copy of their DNA each time they divide.) Men with inherited mutations in these genes have a condition known as Lynch syndrome (also known as hereditary non-polyposis colorectal cancer, or HNPCC), and are at increased risk of colorectal, prostate, and some other cancers.

*HOXB13* This gene is important in the development of the prostate gland. Mutations in this gene have been linked to early-onset prostate cancer (prostate cancer diagnosed at a young age)that runs in some families. Fortunately, this mutation is rare, acquired gene mutations Some gene mutations happen during a person's lifetime and are not passed on to children. These changes are found only in cells that come from the original mutated cell. These are called acquired mutations. Most gene mutations related to prostate cancer seem to develop during a man's life rather than having been inherited, every time a cell prepares to divide into 2 new cells, it must copy its DNA. This process is not perfect, and sometimes errors occur, leaving defective DNA in the new cell. It's not clear how often these DNA changes might be random events, and how often they are influenced by other factors (such as diet, hormone levels, etc.). In general, the more quickly prostate cells grow and divide, the more chances there are for mutations to occur. Therefore, anything that speeds up this process may make prostate cancer more likely.

**1.2.2.5.3.4.3Environmental factor** Possible role for environmental is suggested by the increased frequency of prostatic carcinoma in certain industrial settings and by significant geographic differences in the incidence of the disease.Among environmental factors, a diet high in animal fat has been suggested as a risk factor.

**1.2.2.5.3.5 Diagnosis of prostate cancer**

The main test urine test to rule out urine infection,Prostate specific antigen (PSA), Digital rectal examination (DRE),Prostate biopsy and Gleason score.

**1.2.2.5.3.5.1 PSA value**

Assay of serum level of prostate specific antigen

PSA is a 33 KD proteolytic enzyme produced by both normal and neoplastic prostatic epithelium. PSA value of 4,0ng /l has been used as the upper limit of normal. Cancer cells produced more PSA,prostatitis, nodular hyperplasia also elevate serum PSA level( Vinay *et al*., 2000 ).

PSA velocity These include rate of change of PSA values with time

PSA density Determination of the ratio between the serum PSA value and volume of the prostate gland

Measurement of free versus bound forms of circulating PSA(Vinay *et al*., 2000).

**1.2.2.5.3.5.2Digital rectal examination (DRE)**

By feeling the prostate through the wall of the back passage (rectum)**.**

Normal , a normal size for age with smooth surface ,Large than expected for age that could be sign of an enlarged prostate ,Hard and lumpy this could be a sign of prostate cancer(NCCN2017)

**1.2.2.5.3.5.3Prostate biopsy**

this involves using thin needle to take small sample of tissue from the prostate,The tissue is then look out under microscope to check cancer.There are two main type of biopsy:Trans rectal ultrasound (TRUS) guided biopsy where the needle goes

through the wall of back passage,template (Tran'sperineal biopsy) where the needle goes through skin between testicles and back passage.

#### 1.2.2.5.3.5.4Gleason grade

When cells are seen under the microscope, the have different patterns depending on how quickly they are likely to grow the pattern is given a grade from 1 to 5 this called the Gleason score **,**grade 1 and 2 are not cancer and grade 3, 4 and 5 are cancer.

#### 1.2.2.5.3.5.5Magnetic Resonance imaging (MRI) and computerized tomography (CT)scan

Having MRI, CT scan to find out whether the cancer has spread outside the prostate and where is has spread to.

#### 1.2.2.5.3.5.6Bone scan

Show whether any cancer cells have spread to bones which is common place for prostate cancer to spread to.

#### 1.2.2.5.3.5.7Positron emission tomography (PET) scan

This shows how well different parts of body are working. It can used to check if cancer has spread outside the prostate, it`s normally used to see if cancer has come back after treatment rather than when you are first diagnosed.

### 1.2.2.5.3.6 Staging of prostate cancer:-

Anatomical staging of the extent of disease plays an important role in the evaluation and treatment of prostatic carcinoma. Prostate cancer is staged by clinical examination, surgical exploration,and radiographic imaging

technique.Anatomic feature employed in the revised TNM staging system are summarized in table 1.1 (Vinay *et al*., 2000)

### 1.2.3 MTHFR(C677T) polymorphism and Prostate Cancer

Prostate cancer is the most common malignancy and the second leading cause of cancer-related deaths among men in many countries. The incidence and prevalence account for 15.3% in developed countries and 4.3% in developing countries. Diet, hormone levels, age, and both chemical and carcinogen exposure are the potential risk factors for prostate cancer. Although the etiology of prostate cancer is largely unknown, both genetic and environmental factors might contribute to the development of prostate cancer

Prostate cancer has a long latency period however serum levels of prostate-specific antigen (PSA) showed an early rise by the specific activity of tumor cells, therefore PSA is the major serum marker of prostate cancer. Recently total serum levels of homocysteine has been identified as a marker of prostate cancer

Genetic polymorphisms that alter the activity of the enzymes of biotransformation have been reported to be associated with cancer development and progression. Previous reports revealed that polymorphisms in the MTHFR gene play a critical role in cancer development.

Methylenetetrahydrofolatereductase (MTHFR) is a key enzyme in folate metabolism which results in both DNA synthesis, repair and intracellular methylation reactions

MTHFR regulates the entrance of folates into the methylation cycle. Together with other enzymes MTHFR irreversibly catalyzes the conversion of 5,10-

methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate and the methyl donor for re-methylation processes, which is involved in the homocysteine/ methionine conversion. Methionine is then activated to S-adenosylmethionine, a universal methyl donor in numerous transmethylation reactions including methylation of DNA, proteins, lipids, and synthesis of polyamines. This conversion is critical in controlling intracellular homocysteine levels and maintaining adequate levels of S-adenosylmethionine. MTHFR enzyme function may effect cancer risk in two ways. The impaired MTHFR activity might result in accumulation of 5,10-methylenetetrahydrofolate and therefore a reduction in 5-methyltetrahydrofolate levels which lowers the source of methyl groups for DNA methylation, an important epigenetic process in gene regulation, leading to modifications in DNA conformation and gene expression. In the case of a reduction in the substrate of the MTHFR, 5,10-methylenetetrahydrofolate, the levels of thymidylate biosynthesis are decreased leading to deoxynucleotide pool imbalances and increased uracil misincorporation into DNA which potentially causes to chromosomal damage, DNA double-strand breaks, impaired DNA repair, and DNA hypomethylation.MTHFR gene has 11 exons spanning 2.2 kb .Several polymorphic sites have been identified on MTHFR gene. A common 677 C-T transition in the MTHFR gene is a well identified genetic determinant of hyperhomocysteinemia, and results in a termolabile protein with a decreased enzymatic activity. The C677T variant lies in exon 4 at the folate binding site of the MTHFR gene and results in the substitution of an alanine by a valine (Ala222Val) residue. Some reports have shown an association between MTHFR gene polymorphism with cancer development(Ozlem et al.,2011).

## 1.3 Previous studies

A study done by Ozlem et al in 2011 among 55 case of Turkey patients with prostate cancer and 50healthy men .Polymerase chain reaction (PCR),restriction fragment length polymorphism (RFLP) , and agarose gel electrophoresis techniques were employed to determine MTHFR C677Tmutation. The ferquencies of the CT genotype (p= 0.025) and T allele (p= 0.023)was found to be higher in control subject when compared with patients group (Ozlem *et al* .,2011) .

Mohammed et al., conducted a study in 2009 among Eskisehir population. Their study included 93 prostate cancer patients between the ages 50-89 and control group of 166 benign prostate hyperplasia .C677T and A1298C polymorphism were grouped in tow groups and they do analysis to see if there is statistically meaningful difference.they observed that C677T polymorphism of MTHFR gene produces no statistically significant frequency in prostate cancer patients and male control not having prostate cancer (Mohammed et al.,2009)

## 1.4 Rationale and objectives

### 1.4.1 Rationale

MTHFR gene is located on 1p36.3 and has 11 exons spanning 2.2 kb (cDNA Gen Bank accession number U09806) (Goyette *et al*., 1998; Pereira *et al*., 2006). Several polymorphic sites have been identified on MTHFR gene. A common 677 C-T transition (rs1801133) in the MTHFR gene is a well identified genetic determinant of hyperhomocysteinemia, and results in a termolabile protein with a decreased enzymatic activity. The C677T variant lies in exon 4 at the folate binding site of the MTHFR gene and results in the substitution of an alanine by a valine (Ala222Val) residue.

Venous thromboembolism is complication commonly encountered in cancer patient and considered to be major cause of morbidity and mortality .and the prostate cancer is one of four major types of cancer.

The genetic poly morphsim of thrombophilic factor (MTHFRC677T) in prostate cancer patient have been focused on to determine the effect of MTHFR C677T gene on risk of prostate cancer and risk of thrombosis in prostate cancer patient caused by hyperhomocystenemia as result of MTHFR C677T mutation,

A conflict results have reported regarding the MTHFR gene polymorphism and cancer development. (Frosst *et al*., 1995; Bailey *et al*., 1999; Kimura *et al*., 2000; Stern *et al*., 2000; Heijman et al., 2003; Cicek *et al*., 2004; Krajinovic *et al*.,2004; Signal *et al*., 2004; De Mattia *et al*., 2009).

### 1.4.2 Objectives

#### 1.4.2.1General Objective

To study the associations between the thrombophilic mutation of MTHFR C677T polymorphism and prostate cancer in Sudanese patients

#### 1.4.2.2 Specific objectives

1. To determine the genotypic variant of methylene tetrahyhrofolatereductase (MTHFR) C677T polymorphism in patients with prostate cancer using PCR

2. to correlate the genotypic variant with patients demographic data.

# Chapter Tow

# Materials and Methods

## 2.1 Study design

It is observational analytical case control study approved by Sudan university of science and technology faculty of medical laboratory science.

## 2.2 Study area and period

This study was carried out during period from June to December 2017 at Taiba cancer center and Khartoum center for oncology

## 2.3 study population

Sudanese patients diagnosed by histopathology with prostate cancer were recruited to participate in this study as well as apparently healthy volunteers were enrolled as control group

### 2.3.1 Inclusion criteria

Sudanese patients diagnosed with prostate cancer.

## 2.4 Sample size

The sample was collected from 38 prostate cancer patients as (case) and 40 healthy individual as (control).

## 2.5 Ethical consideration

The consent was taken from participant to obtain their remnant sample.

## 2.6 Data collection

Data was collected from hospital record. .

## 2.7 Sample collection

2.5 ml of venous blood sample was collected from 38 patients and from 40 healthy volunteers for PCR performance

## 2.8 Methodology

### 2.8.1 DNA extraction by salting out method:

#### 2.8.1.1 Procedure

300 µl of blood sample was Placed in 1.5 ependorf tube, 1000 µl RCLB was added to the tube, Mixed well, centrifuged at 2500 rpm for 10 minutes, Supernatant was discarded and the pellet (WBCs) washed again with 1000µl of RCLB (repeated until clear pellet was obtained). WCLB, 10µlprotinase K and 10 µl SDS were added to the clear white pellets. The mixture was incubated for 2 Hours at $56^0$C.100 µl of 6 M NaCL was added to precipitate the protein and mixed well by vortex. 200µl of ice cold chloroform were added to tube and centrifuged at 12000 rpm for 6 minutes.The aqueous phase was transferred carefully to clean ependorf tube, and to which double volume of cold absolute ethanol was added to precipitate the DNA. The tube was centrifuged at 12000 rpm for 5minutes.The supernatant was poured off without disturbing the precipitate and then washed with 600µl 70% ethanol. The tube content was centrifuged at 7000 rpm for 5 minutes, the ethanol was discarded and the tube was left to air dry.The pellets were re suspended in 50µl of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 and leaved to dissolve overnight.

### 2.8.2 Determination of DNA quality and purity

Part of the DNA solution was mixed with loading dye 5 in 1 and DNA quality and purity was determined using gel electrophoresis.

DNA was transferred into 1 ml eppindrof tube and preserved at -20°C until

PCR is performed.

### 2.8.3. Detection of MTHFR C677T polymorphism

All patients with prostate cancer and control were examined for the C677T polymorphism using allele specific PCR.

**Table 2.1: The primers sequence used were as follow:**

| Primers | Sequence | CC | TT |
|---|---|---|---|
| MTHFR-T | 5' – GCACTTGAAGGAGAAGGTGTCTGCGGGCGT3' | 170bp | |
| MT MTHFR-C-polyG | 5'GGCGGGCGGCCGGGAAAAGCTGCGTGATGATGAAATAGG-3 | 150bp | 50bp |
| MTHFR-cf, | 5' - CCATGTCGGTGCATGCCTTCACAAAG-3' | | |

**Table 2.2:PCR cycle** :

| Profile | Temperature | Time duration | Number of Cycles |
|---|---|---|---|
| **Initial Denaturation** | 94°C | 10 minutes | 1 |
| **Denaturation** | 94°C | 30 seconds | 35 |
| **Annealing** | 60°C | 30seconds | |
| **Extension** | 72°C | 30 seconds | |
| **Final Extension** | 72°C | 10 minutes | 1 |

**Table 2.3: master mix tube preparation:**

| Reagents | Volume |
|---|---|
| Double D.W | 15µl |
| MTHFR-T | 1 µl |
| MT MTHFR-C-polyG | 1 µl |
| MTHFR-cf, | 1 µl |
| Template DNA | 2 µl |
| Total reaction volume | 20µl |

### 2.8.4 Demonstration of PCR product:

Five µl of the PCR product (ready to load) was electrophoresed on 1.5% agarose gel, and was stained with ethedium bromide, 1X TBE buffer was used as a running buffer. The Voltage applied to the gel was 100 volt with time duration of 30 minutes. 50 bp DNA ladder was used as molecular weight marker with each batch of samples .Finally, PCR product was demonstrated by gel demonstration system**.**

### 2.9 Data analysis

The data were analyzed using the SPSS computer program version 16. Chi square and was done to determine the association and risk factor.

Interpretation

# Chapter Three

## Results

The participants included 78 men subjects, 38 of them had a prostate cancer their age range was 44-67 years and 40 were age matched apparently healthy volunteers as control group.

The distributions of genotypes and alleles of MTHFR C677T are shown in Table 3.1. The frequencies of CC and TT genotypes among the patients with prostate cancer were 95 % and 5% respectively, and among the control subjects 97.5 %, and 2.5%, respectively. When the MTHFR 677CCgenotype was defined as the reference, the OR for the TT genotype was 2.1053 (95%CI: 0.1833 to 24.1832, P= 0.5500). There was no significant association between cases carriage any of this mutation and risk of thrombosis on prostate cancer patients.

Table( 3.1) Genotype distribution in study groups.

| Genotype | Patients (%) | Controls (%) | P-value | OR (95%CI |
|---|---|---|---|---|
| Normal homozygous C/C | 95 | 97.5 | 0.5500 | 2.1053 |
| Mutatnt homozygous TT | 5 | 2.5 | | |

# Chapter Four

## Discussion, Conclusion and Recommendations

### 4.1 **Discussion**

The association between MTHFR C677T polymorphism and prostate cancer has been investigated in several previous studies and different conclusions have been reported. Heijmans BT *et al* reported a positive association of MTHFR C677T polymorphism with prostate cancer( Heijman et al., 2003), whereas Kimura F et al. found a weak positive association with higher tumor grade(Kimura *et al*., 2000). Cicek et al. reported a reduced risk of prostate cancer progression with C677T allele (Cicek et al., 2004). In addition Reljic *et al* reported no any effect of the MTHFR C677T variants on prostate cancer (Reljic et al., 2007). Sadewa *et al* study included 40 prostate cancer patients, their MTHFR C677T genotype frequencies and haematological characteristics were determined and compared with 40 age matched normal subjects as control. The frequency of the C677T mutation differs among ethnic populations. The T allele frequency ranges from 0.06 to 0.59 and the frequency of the T/T genotype (homozygosity for the C677T mutation) ranges from 0.00 to 0.35 among ethnic populations (Sadewa et al., 2002) . Our study showed low frequency of mutant MTHFR C677T genotype with 5 %TT genotype (homozygote) & 0% CT (heterozygote) our finding is similar with previous reports among different study population in Africa (Franco, et al., 1998, Annichino-Bizzacchi et al., 1998). Our study showed a statistically insignificant association between MTHFR C677T polymorphisms and the risk of prostate cancer with 2.1 folds increased risk. Low impact of MTHFR C677T polymorphism as a risk factor in prostate cancer, in the study population, may be attributed to the low frequency of the mutant genotypes, in particular 677 TT, rather than the actual contribution in the pathogenesis of prostate cancer. The present study included a relative small

sample of patients. The assessment of homocysteine level and folic acid has not been performed, representing another limitation of our study.

## 4.2Conclusion

No significant variations in MTHFR C677T genotype distribution among males, who suffered from prostate cancer and control group when compared with.

There was no statistically significant difference in genotypes distribution when compared in patients with prostate cancer and control group.

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**4.3 Recommendations:**

Another study should conducted to evaluate the role of MTHFRC677T polymorphism in thrombotic complication among patients with assessment of homosestine level.

Further studies with large sample size and patients demographic data should be taken to correlate the genotypic variant with patients demographic data.

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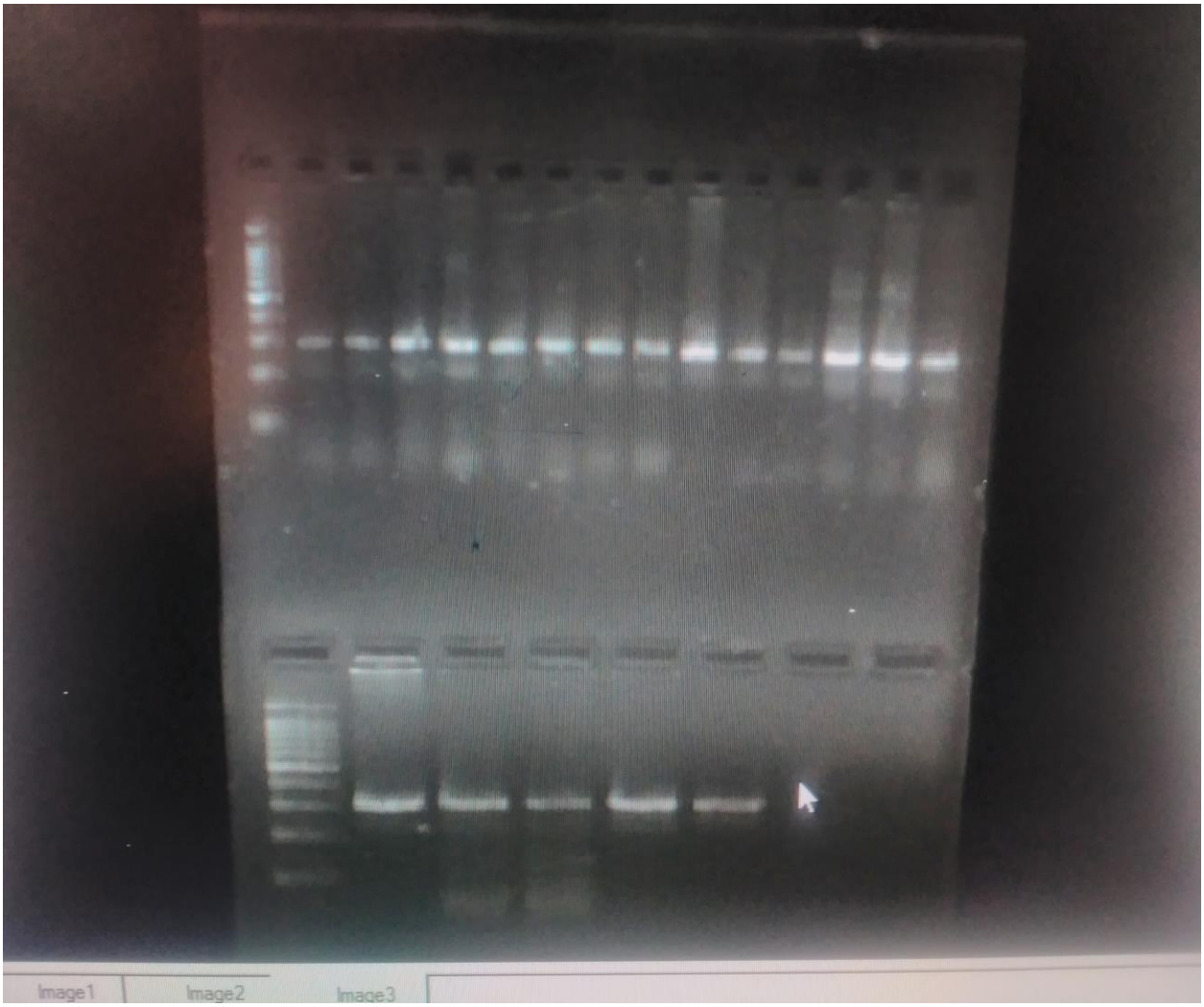
# Appendices

Appendix (1)

Amplihed fragment  of MTHFR gene



Appendix (2)

PCR machine