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SYSTEMATIC REVIEW ON DIAGNOSTIC APPROACHES IN MALE REPRODUCTIVE TRACT DISORDERS

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ABSTRACT

The male reproductive system consists of the internal structures such as testes, epididymis, vas deferens, prostate and the exterior structures such as the scrotum and the penis which facilitate the production, storage, and ejaculation of sperm for the purpose of fertilization, as well as to create essential androgens for the maturation of males. In the recent years, the prevalence of male-specific reproductive disorders has sharply increased that pose a substantial health risk. The clinical influence of the male reproductive disorders cannot be ignored as they can lead to serious conditions such as preterm birth, increased susceptibility to sexually transmitted infections (STI), infertility and cancer. Clinicians currently rely on semen analysis for the diagnosis of male factor infertility, although semen analysis alone is usually insufficient for diagnosis. Also, the study and treatment of male reproductive disorders has entered a new era as a result of developments in molecular technology such as digital PCR, multiplex real-time PCR, high throughput sequencing, and new generation sequencing. However, diagnosis at an early stage is crucial for the prevention of underlying pathologies has led to a soaring demand for novel diagnostic tools, such as protein biomarkers, which allow for better and earlier treatment of various genital tract pathologies. This review, highlights the disorders and outline of diagnostic tools for male reproductive system.

KEYWORDS: Male reproductive tract, Diagnostic tools, Genetic markers, Protein markers.

INTRODUCTION

The male reproductive tract is a network of external organs, internal organs, accessory glands and genital ducts that play a vital role in the production, maturation and transportation of sperms and male sex hormones. The male external genitals include a penis and scrotum. The internal genital organs include testis, epididymis, vas deferens and urethra. Male sex accessory glands consist of the paired seminal vesicles, single prostate gland and small paired bulbourethral glands. Male genital ducts consist of ductuli efferentes, ductus deferens and ejaculatory duct.[1]

1. EXTERNAL ORGANS

1.1 Penis: Penis, the male external genitalia consist of two pairs of cavernous corpuscles enclosed in two layers of the tunica albuginea and elastic connective tissue sheath. Orientation of the collagen bundles in the outer tunica determines the thickness and strength of the tunica. Smaller fibres in the inner layer create the septum of the penis when they meet in the median plane, where they are organised in a circular pattern. There are perpendicular intracavernous pillars that originate at roughly six-o'clock and insert into tunica on the cavernous bodies' lateral walls that help to strengthen the tunica albuginea structure. Corpora cavernosa contains erectile tissue that makes up most of the penis.[2] Intercourse and urine discharge are the two primary tasks that are performed by the penis.[3]

1.2 Scrotum: Scrotum is a thin external sac of skin made up of smooth muscles (dartos fascia) that is placed under the penis and is divided into two compartments; each compartment comprises one of the two testes and one of the epididymis. The scrotum has a usual wall thickness of 8 millimeters where both parietal and visceral layers are found. The testes and epididymis are protected by the visceral layer, whereas the parietal layer covers the inner surface of the scrotal wall. Spermatozoa, testicles, epididymis and spermatic cord are all found in the scrotal sac. The function of the scrotum is to shield the testes so as to maintain the temperature several degrees below the normal body temperature. The relatively cool temperature of the scrotum is thought to be important for the production of viable sperm.[4]

2. INTERNAL ORGANS

2.1 Testis: Testes are responsible for the production of sperms and testosterone. Each Testis has an oval shape with a volume of 15–22 cm^3 and a longitudinal length of 4.5–5.1 cm on an average. The outside of each testis has a serous membrane covering of tunica vaginalis,

underneath which is a dense covering of tunica albuginea. Testis comprises of 250 compartments called testicular lobules divided by septae. They further consist of numerous coiled seminiferous tubules, each of which contains germ cells and other interstitial tissues. Within the seminiferous tubules, the stem cells are present which divide and differentiate into spermatozoa via spermatogenesis.[5]

2.2 Epididymis: Epididymis consists of a coiled tube walled around the edges of testes where the developed spermatozoa mature. There are three primary sections in the epididymis; the caput, corpus, and cauda. Spermatozoa enter the epididymis via the head or globus major that covers the upper portion of the testis (caput epididymis), progress to the body (corpus), and finally enters the tail or minor globus (cauda), where they are stored.[6] Throughout their passage via the epididymis, maturation of spermatozoa occurs as to acquire the motility required for an egg fertilization.

2.3 Vas deferens: Vas deferens, originating from the mesonephric ducts, is a muscular tube that connects the epididymis to the ejaculatory ducts to transport sperm. The initial section of vas deferens known as the convoluted component begins at the cauda epididymis. It travels from epididymis organ to the back of the prostate where it joins with one of the two seminal vesicles. Consequently, the vas deferens serves as a conduit between the testis and the secondary reproductive tissues (such as the prostate and the seminal vesicles) in the pelvis.[7]

2.4 Urethra: Urethra is a narrow fibromuscular tubular structure containing the smooth muscles, connective tissue and the submucosa with collagen fibres and micro vascularization. An anterior (distal) portion includes the bulbous and penile segments. The posterior portion comprise the prostatic and membranous segments. The urogenital diaphragm and the membranous urethra meet at the bladder neck where the prostatic urethra extends to a length of 3 to 4 cm. The major role of male urethra is to connect the urinary bladder to the penis and also, as a conduit for semen and sperm during sexual acts which is under the control of bulbospongiosus muscle present in the urethra.[8]

3. MALE ACCESSORY GLANDS

3.1 Seminal vesicles: The seminal vesicles are the paired saccular glands which are present posterior to the prostate gland and are approximately 5cm long. Approximately two-thirds of the ejaculate is produced by the seminal vesicles, which act as secretory glands. The seminal vesicles add around 60% of the semen volume. The seminogelin proteins further provide the thickness to semen which is required for coagulation upon ejaculation. The components of the vas deferens and the seminal vesicles blend together and are further transported to the prostate gland.[9]

3.2 Prostate gland: The prostate, just like seminal vesicles, is only found in men's reproductive system and contributes to the ejaculate's secretion. The size of the prostate gland is of the walnut and it secretes alkaline milky liquid to the seminal fluid called semen. The prostate develops from the primitive posterior urethra's buds and spreads over the surrounding mesenchyme. Three distinct lobes are formed from the primitive buds: (1) huge right and left edgewise lobes, (2) medium-sized anterior and posterior lobes, and (3) a little middle lobe. The ejaculatory ducts pass through the prostate's central zone on their way to the verumontanum, which is located at the base of the prostate's ejaculatory duct system.[10] This gland adds proteases to the semen which helps in the liquefaction facilitating the release of sperm.

3.3 Bulbourethral gland: Bulbourethral gland is also referred as Cowper’s gland. It is located under the prostate gland which secretes a thick fluid. As its secretion enters the penis through an inconspicuous opening in the urethra's distal portion, it helps to lubricate the passageway. Both the external urethral sphincter's fibres and the membrane urethra surround the bulbourethral glands, which are small, spherical, yellowish pea-sized structures (1 cm in diameter).[11]

4. MALE SEX ACCESSORY DUCTS

Male sex accessory ducts are vital part of the male reproductive system, which includes the ductuli efferentes, ductus deferens and ejaculatory duct. The sperms are collected from the testes by the ductuli efferentes and further conducted by ductus deferens to the ejaculatory duct which expels them by strong contractions of the smooth muscles.[12] Males have numerous severe anatomical and functional abnormalities such as cryptotorchidism, testicular and prostate cancer, semen abnormalities (azoospermia and oligozoospermia) as well as other male-specific disorders such as varicocele, peyronie’s disease, epididymitis, orchitis, in the abovementioned reproductive organs which leads to the impotency and reproductive failure.

5. Disorders of the male reproductive tract

5.1 Cryptorchidism: Cryptorchidism is a congenital anomaly which is characterised by the failure of one or both testicles from the abdominal cavity further into the scrotum.[13] Less than 1 % of the population i.e., newborns older than three months are affected with cryptorchidism. The prevalence of cryptorchidism in fully-term newborn males is reported to be 2–5%. In fifty % of these boys, the testicles will voluntarily descend into the scrotum during the first three months of life, while only 1% of boys will be born with cryptorchidism. In prematurely born boys, roughly one third of boys will have testicles that have not been descended by the time they are delivered. However, approximately 80 % of boys who were born with undescended testicles will have descended testicles by the third month of life.[14]

Although the exact origin of cryptorchidism is still unknown but there are several uncommon diseases that result in undescended testes, suggesting a multifactorial basis of cryptorchidism. Prune belly syndrome, posterior urethral valve, and abdominal wall defects are all associated with an increased frequency of cryptorchidism.[15] Birth-related conditions such preterm delivery, low birth weight, unusually low levels of maternal oestrogen, and placenta insufficiency are thought to increase the likelihood of developing cryptorchidism.[16]

In addition to a variety of complicated syndromes that include midline and caudal anomalies, there is a wide range of genetic and hormonal abnormalities that may lead to cryptorchidism. Additionally, environmental factors and lifestyle choices may also have a negative impact on human testicular descent.[17]

In patients with isolated cryptorchidism, abnormalities of the hypothalamic-pituitary-gonadal axis and testosterone biosynthesis have been reported more frequently in contrast to genetic defects like 5'-reductase deficiency, HOXA10 or INSL3 gene mutations, polymorphisms of the oestrogen receptor alpha gene, androgen-receptor mutations and polymorphisms of the CAG repeat of the androgen receptor.[18]

Cryptorchidism is known to be associated with malignancies of the human male testis. Even in cases when cryptorchidism is detected and treated promptly and effectively, it is likely that the condition will have long-term repercussions, such as decreased fertility, depression, and an increased risk of testicular cancer.[19]

In individuals born with cryptorchidism, spermatogenic tissue degeneration and decreased spermatogonia counts were detected as early as the second year of life. Unilateral as well as bilateral cryptorchidism limit fertility. 10%-20% of patient populations with unilateral cryptorchidism and 40% to 80% of patients with bilateral cryptorchidism suffer from infertility. Multiple factors contribute to the lower fertility of males with cryptorchidism. Orchiopexy, which is performed to correct bilateral cryptorchidism, is associated with a fertility loss of at least 38 %.[20]

It is reported that the incidence of cancer in adults with childhood cryptorchidism is found to be five to ten times higher than usual. The rise in cryptorchidism rates appears to coincide with the rise in prevalence of testicular cancer.[21] Because cryptorchidism is more typically found in people on the right-side of the male reproductive system, TC is also a right-sided disorder. Cryptorchidism has a risk ratio ranging from 2.5 to 14 for the occurrence of testicular cancer.[22]

5.2 Testicular cancer: Germ-cell neoplasms account for 95% of all testicular tumours. Seminomas, embryonal carcinomas, malignant teratomas, and choriocarcinomas are the four distinct forms of germ-cell tumours recognised by (International Agency for Research on Cancer) IARC.[23] These lesions are classified into two types based on their histology: seminomas and nonseminomas. Seminomas account for about half of all germ-cell tumours, with the other half being nonseminomas. Nonseminomas are often mixed histological tumours that might have a range of seminoma as well as nonseminoma histological subtypes. A recent research that looked at the histopathology of mixed germ-cell tumours (MGCTs) discovered a significant link among teratomas and yolk sac tumours.[24]

The discovery of testicular germ cell tumor (TGCT) risk factors provided insight into the pathobiological processes behind the development of testicular cancer. DNA demethylation, which includes the erasure of genomic imprinting, is usually followed by demethylation and meiosis, which causes embryonic germ cells to lose their totipotent character and become into fully committed germ cells. However, the risk factors that have been reported for TGCTs suggest that pre-GCNIS or totipotent GCNIS50 is caused by a disruption in the naturally occurring phenomena of the spermatogonial niche. This disruption can either result in the death of germ cells and subfertility or infertility, or it can result in a delay in the maturation of gonocytes into spermatogonia.[25]

All three forms of TGCTs may take on the appearance of a somatic, totipotent, or the lineage of a GCT which is thought to be governed by the developing capacity of the cell of origin as well as a variety of recurring processes. Polyploidization and aneuploidization are the earliest pathogenetic steps in type II and type III TGCTs, respectively, followed by chromosomal imbalances and, maybe later, uncommon somatic mutations.[26]

Indeed, when invasive lesions arise, somatic mutations appear to favour development of these mutations may contribute to the genetic variability of TGCTs. In summary, genome duplicacy and chromosomal imbalance seem to be a common mechanism in the genesis and progression of human TGCTs.[25]

Testicular cancer has a clear age distribution. Between the age groups of 25 and 35, the incidence peaks. After the age of 80, considerably smaller peak appears. In contrast to the majority of other cancers, which often heighten considerably later in life, this disease has a distinct age distribution.[27] Testicular cancer incidence seems to be correlated with sex hormone activity. The incidence of testicular cancer varies with race. Compared to Caucasian populations, the incidence of testicular tumours in Blacks and other non-white races is exceptionally low.[28]

5.3 Prostate cancer: Approximately 40% of all malignancies in males are caused by prostate disease.

Cancer deaths from prostate cancer continue to climb and pose a major health burden, particularly for those living in the Western world. It is the most often diagnosed cancer in men in the United States, potentially as a result of widespread screening for asymptomatic men.[29]

The endocrine system, age, and race all appear to play a role in the development of prostate cancer. Men's reproductive ages, races, and hormone levels all play a role in the prevalence of prostatic tumors. Prostate cancer becomes more likely with age and is more common in some races. The implications of age or gender are well recognized.[30]

Prostatic glands are divided into two major groups: the inner and outer. The inner glands of the prostate are responsible for the growth of the prostate. Prostatic cancer originates in the outermost glands of the prostate. In patients with early-stage, prostate cancer often has no symptoms at all. Benign prostatic hyperplasia (BPH) related symptoms of the lower urinary tract may be present in prostate cancer patients including a weak stream, hesitancy and urgency as well as frequency and nocturia, straining, intermittency and incomplete emptying. Both benign prostatic hyperplasia and prostate cancer are common in older men. Both disorders appear to be androgen-dependent for growth, although benign prostatic hyperplasia occurs in the prostate's centre or transitional zone and cancer in the gland's periphery. Hematuria, hematospermia, and erectile dysfunction should all be considered in men who exhibit these symptoms (ED).[31]

5.4 SEMEN ABNORMALITIES

Semen abnormalities are basically divided into azoospermia and oligozoospermia.

5.4.1 Azoospermia: Azoospermia is a condition or infertility disorder which is characterized by the presence of no sperm at all in the semen. It is diagnosed in around 1% of all men and in 10% to 15% of individuals who are infertile. Azoospermia can be categorized as either obstructive azoospermia (OA) or non-obstructive azoospermia (NOA), both of which have quite different causes and treatments. OA is less common than NOA. Approximately 40% of males with azoospermia have OA. The most common cause of OA is the existence of a blockage that compromises the genital tracts patency at one or more places along the male reproductive system. Common examples include genitourinary infections that cause obstruction, congenital bilateral absence of the vas deferens (CBAVD), which is linked to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene etc.[32] Non-obstructive azoospermia may be induced by a mechanism inherent to the testis, known as primary testicular failure, which results in decreased sperm production. In addition to non- obstructive azoospermia, endocrine disorders can produce secondary testicular failure, commonly known as aberrant spermatogenesis.[33]

Although there are a number of factors that may lead to azoospermia, but the testicular etiologies may be broadly categorized into three groups: pretesticular, testicular, and post-testicular. Endocrine disorders that have a negative impact on spermatogenesis are the most common pretesticular causes of azoospermia. Testicular etiologies are characterized by intrinsic abnormalities of spermatogenesis that occur inside the testicles. Post-testicular azoospermia may be caused by obstruction at any location in the ductal system of the male reproductive tract. Treatment for the pre- and post-testicular disorders that lead to azoospermia is often possible, which could make it easier to restore reproductive potential. In contrast, testicular problems are often permanent, and therapies linked to intrinsic testicular abnormalities have far lower success rates.[34]

It is estimated that around 29 % of males who are azoospermic have underlying genetic problems. Chromosomal or gene mutations (whether nuclear or mitochondrial) as well as epigenetic changes are examples of common genetic disorders. Chromosomal abnormalities may affect either an individual autosome or their sex chromosomes. The most prevalent cause of azoospermia is Y chromosomal microdeletions, which may be identified in anywhere from 5 % to 15 % of infertile males who have azoospermia. The most common chromosomal disorders affecting 10–20% of azoospermic males are Robertsonian translocations and Klinefelter syndrome (KFS).[35]

Infertility affects 15% of couples. Nearly 1% of males and 10 to 15% of infertile men have azoospermia. In the US, 600,000 males of reproductive age suffer from non-obstructive azoospermia. Azoospermic males have a higher cancer risk than others. 5-8% of testicular cancer patients are azoospermic.[36]

5.4.2 Oligozoospermia: There are three levels of oligozoospermia: mild, moderate, and severe. Mild is described as having less than 15 million sperm in the ejaculate, while moderate has between 5 and 10 million sperm, and severe has less than 5 million sperm in the ejaculate. There are many similarities between the condition of oligozoospermia and azoospermia, therefore it can be described a less intense form of the condition. Oligozoospermia-specific gene deletions have been seen in some cases. Some people may experience severe oligozoospermia as a result of the same.

5.5 Varicocele: Varicocele is the male reproductive disorder associated with the abnormal enlargement and dilation of the pampiniform vein which originates in the testis and houses the testicular veins as well as channels blood from each testicle. Approximately 15% to 20% of the all the men have varicoceles, but around 40% population of the infertile males suffer from varicocele.[37]

Multiple factors are suspected to be at play in the development of varicocele. The testicular vein emerges from the spermatic cord in the inguinal canal and proceeds to the abdomen via the inguinal canal. The right internal spermatic vein connects directly to the low-pressure inferior vena cava on the right side and connects to the left renal vein relatively under high-pressure on the opposite side.[38] Incompetent venous valves causing retrograde of venous blood and raised hydrostatic pressure, as well as anatomic distinctions in venous drainage between both the left and right inner spermatic veins, are the most frequently stated hypotheses for varicocele development.[39] Physical activity during adolescence has been linked to the formation of varicoceles, although physical activity at a later age may make the disease worse but does not affect the overall prevalence of varicoceles in a population.[40]

Varicocele-related problems with sperm generation, shape, and function remain a mystery despite numerous hypothesis. Varicose vein stasis causes leukocyte activation, trapping, and production of ROS (reactive oxygen species) from the activated white cells, which results in (oxidative stress) OS.[41] Stasis, bilateral testicular ischemia, tissue hypoxia, and OS are associated with impaired venous drainage in sub-fertile males with varicocele, all of which may affect the function of the testicles.[42] Varicoceles are clinically relevant as they are the most common cause of poor sperm morphology, low sperm count, impaired motility and abnormal semen analysis. In varicocele individuals, spermatogenesis and testes are negatively affected by heat stress on the testicles. Inhibition of spermatogenesis might be the result of an imbalance in the hormone testosterone. Reduced HSP (heat shock protein) expression and elevated inducers of apoptosis are found in patients with varicocele, resulting in spermatozoa that are vulnerable to apoptosis. In men with varicocele, testosterone levels have been found to be lower than normal [43]. Male infertility can be linked to varicoceles, but the link is not conclusive. Barfield, from the late 1800s, was the first to notice this connection, which was later corroborated by many in the early 1900s.[44]

5.6 Peyronie's disease: Peyronie's disease affects middle-aged males in an idiopathic manner. Tunica fibrous thickening originates as a region of vasculitic inflammation next to the albuginea and may expand further into intercavernosal septum. One or numerous fibrous plaques form as a result and they can calcify, making them apparent on x-rays. In addition to the painful erections, penile deviation, and poor-quality erection distal towards the affected area, Peyronie's disease might lead to an inability to ejaculate.[45]

5.7 Epididymitis: An infection or inflammation of the epididymis, which is a tubular structure found on the posterior and superior part of the testis, is known as epididymitis. Epididymitis affects roughly 600,000 men in the US each year.[46] In 2002, epididymitis accounted for 0.69 % of all outpatient visits by men of age group 18–50. Posterior testicular discomfort in men & boys with epididymitis is often gradual in commencement, unilaterally, and occasionally transmits to the lower abdomen in those with the condition. Epididymitis initially damages the epididymis tail and subsequently extends to influence its head and body. Testicular torsion can be mistaken for epididymitis, which is a common but often misdiagnosed illness.

Epididymitis-causing bacterial pathogens are significantly age- and sexual practice-dependent. *Escherichia coli* is the most common cause of epididymitis in boys under the age of 14 and men over 35. When bacteriuria is caused by benign prostatic hyperplasia, bacteria enter the epididymis and causes it to become infected. Nonspecific bacterial epididymitis, which can be caused by a wide range of aerobic bacteria, can also be attributed to anatomical anomalies.[47] *Chlamydia* and *Neisseria gonorrhoeae* are the most frequent organisms in sexually active men between the ages of 14 and 35 and in elderly men who have intercourse with men. Men who engage in insertive anal sex run the risk of contracting both sexually transmitted bacterial pathogens like *Chlamydia* and *E. coli*, which are coliform bacteria. Some other bacteria, such as *Klebsiella pneumoniae*, *Ureaplasma urealyticum*, *Proteus mirabilis*, have been linked to epididymitis.[46]

Unilateral discomfort and swelling in the scrotum are the most common symptoms of acute epididymitis. The age of a person has a significant impact on the aetiology of disease. Although the exact aetiology is unknown in males fairly younger than 14 years old, anatomical anomalies that cause the reflux of infectious or sterile urine in the ejaculatory ducts have been suggested. Adenoviruses, *Mycoplasma pneumoniae* and enteroviruses, are the most common causes of epididymitis in this age range. Children between the ages of two and eleven may develop a severe scrotum as well as bilateral vasculitis as symptoms of Henoch-Schonleinpurpura.[48]

Chronic epididymitis is characterised by scrotal, testicular and epididymis pain or discomfort that has persisted for at least six weeks. Chronic epididymitis's emergence has been attributed to a number of different etiological reasons such as inflammation, infection, and obstruction of the urinary tract.[49] Patients with chronic epididymitis are more likely to have several sexual partners, frequent use of STD protection, and report musculoskeletal, neurologic, and infectious and/or inflammatory health issues compared to men without this condition.[50]

Infectious chronic epididymitis is frequently caused by a granulomatous response, such as tuberculosis. Patients with granulomatous diseases are more likely to develop chronic infectious epididymitis. In men with a history of or recent exposure to tuberculosis, *Mycobacterium*

tuberculosis (TB) is by far the most frequent granulomatous illness of the epididymis.[51] Chronic noninfectious epididymitis is often known as orchialgia or epididymalgia. *Bacillus* Calmette–Guerin infusions can also cause chronic epididymitis. Sarcoidosis and other factors that cause of epididymitis with granulomatous inflammation are rare.[50]

Any infection or inflammation from the epididymis may extend to the testis itself because of its close proximity, resulting in a disease known as epididymo-orchitis. There are around 20% - 40% of occurrences in which orchitis develops as a result of the direct transmission of an infection, resulting in testicular enlargement and discomfort.[52]

5.8 Orchitis: The inflammation of the testis often known as Orchitis, is almost always a unilateral condition. The symptoms of orchitis may range from mild to severe, and they are often unilateral. Orchitis occurs in 14% to 35% of males infected with the mumps virus.[53] Young people aged 15 to 29 are at the greatest danger. Prepubescent males are rarely diagnosed with orchitis.

Blood-borne dissemination is the main mode of transfer of infection to the testicles. Viruses are considered important pathogens. Pre-existing epididymitis, unprotected sexual intercourse, several sexual partners, prolonged use of a catheter, restriction of the bladder outlet, structural abnormalities, and absence of MMR vaccination are common risk factors. Orchitis linked with systemic (most usually viral) illnesses has been attributed to the blood-borne spread of microorganisms by retrograde ascension of pathogenic bacteria such as *C. trachomatis* or *E. coli*.[54]

Orchitis is the most prevalent extra-salivary inflammatory disease and a significant cause of male infertility. In addition to the typical excruciatingly enlarged parotitis in infected men.[55] A few days to a week after the commencement of parotid swelling, the symptoms usually appear, however orchitis can occur prior to or without parotid swelling.[56]

A week following the commencement of parotitis, mumps orchitis usually reveals itself. There are presently few cases of mumps orchitis in children under the age of 10. Orchitis often develops one to two weeks following parotitis. 30–50% of damaged testicles have a degree of testicular atrophy. The virus affecting the testicular glands during the first few days of infection, causes parenchymal inflammation, separation of seminiferous tubules etc. [57]

Typically, only one testis is affected, and epididymitis occurs in conjunction with orchitis in the majority of instances. It is important to note that inflammatory diseases of the epididymis and testis ('epididymo-orchitis') can occur simultaneously.

It is well acknowledged that male reproductive tract infections and inflammation have a role in infertility. Orchitis and epididymo-orchitis caused by local or systemic infection, as well as noninfectious aetiological causes, are of special importance because of their influence on male reproductive function.

6. DIAGNOSTIC TOOLS FOR MALE REPRODUCTIVE DISORDERS

In order to diagnose male reproductive disorders, medical science has devised a range of tests to aid in diagnosis. An accurate diagnosis of the causes of male reproductive failure is obligatory in order to plan for and implement appropriate, proven treatment strategies. Discovery of a remedial reason is desirable and various medical therapies can hopefully be employed to restore natural fecundity. In 1978, the WHO commissioned a task force to provide a consensus statement for the diagnosis of male disorders, especially infertility. In the present era, from basic semen analysis to novel molecular diagnostic modalities have taken a major account in the diagnosis of male disease entities, as discussed below.

6.1 Semen analysis: Semen analysis is a lab test that evaluates sperm count, motility, and morphology. This test measures the functionality of numerous male reproductive glands and organs. Macroscopic examination involves the observation of the colour, opalescence, volume, pH, consistency and odour of the semen. The test sample that is very transparent or translucent is azoospermic or has a very low sperm content indicating severe oligozoospermia. A red or brownish hue may signal blood or an illness (hematospermia). A yellowish hue suggests a high quantity of flavoproteins that is a possible indication of active infection and presence of inflammatory cells in the sperm. Microscopic examination of the semen involves the observation of the sperm motility, viability, liquefaction of the semen, agglutination of sperm, detection of the round cells and cellular debris in the semen, aggregation of sperm, morphology of sperm, etc.[58]

6.2 Biochemical tests of seminal fluid: Over the years, the released biomolecules in seminal fluid have served as diagnostic biomarkers for male reproductive system diseases. In semen, biochemical compounds of secretory components from the prostate, seminal vesicles, and epididymis provide information on the functional status of these organs. These indicators include fructose as a marker for the seminal vesicles, zinc / acid phosphatase as a marker for the prostate, and carnitine as a marker for the epididymis, etc.[59]

A low zinc concentration may be an indication of a diminished secretion by the prostate, which may be caused by an ongoing case of prostatitis or another inflammatory illness of the prostate. A very low fructose level, a low semen volume, and a low pH are all

indicators of obstructive azoospermia.[58]

6.3 Endocrine evaluation: The integration of hypothalamic-pituitary-testicular axis is necessary for male reproductive activity. Hypogonadism and infertility may come from a disruption of endocrine functionality at any level. The measurement of blood LH, FSH, and testosterone is necessary for the clinical and laboratory assessment of the endocrine function of the reproductive hormonal axis (hypothalamus, pituitary and testes).[60] Endocrine evaluation is a great tool for the checking of – (1) aberrant testicular size and/or disease; (2) abnormal semen parameters; (3) reduced sexual function; and (4) other clinical abnormalities suggestive of a particular endocrinopathy.[61]

6.4 Imaging: The primary use of imaging is to identify treatable factors of fertility problems, such as congenital disorders and conditions that obstruct sperm transportation. Sonography and magnetic resonance imaging (MRI), together with invasive methods including venography and vasography, are the imaging modalities typically used to examine the male reproductive system.[62] The most recent technological advancements in terms of quality of image and resolution have made ultrasound (US) a popular diagnostic tool. The evaluation of male reproductive disorders now frequently involves the use of ultrasound (US), which creates images through the use of high-frequency sound waves. US is crucial in the identification of malignancy or in the monitoring of the cryptorchidism and the contralateral testes.[63] The most commonly used and highly regarded technique for assessing varicocele is Color Doppler ultrasonography (CDUS). The physical examination is still the primary method of managing varicocele, however, CDUS provides a greater level of diagnostic precision.[64]

6.5 Testicular biopsy: Testicular biopsy is considered as a cornerstone of diagnosis from many years for diagnosing various male disorders. A testicular biopsy is the primary diagnostic procedure used to differentiate between obstructive and non-obstructive azoospermia. The ability to distinguish between normal and pathological testicular cell types is assisted by histological investigation and meiotic chromosomal studies. These two methods provide useful qualitative as well as quantitative information.[65] In recent years, the advent of assisted reproductive technologies, particularly intra-cytoplasmic sperm injection (ICSI), has led to the evolution of the testicular biopsy from a basic routine examination test to a rigorous therapeutic tool.[66]

6.6 Emergence of molecular diagnostic tools: Unfortunately, the existing laboratory tests still fail to provide more robust, specific and sensitive data that could contribute in the diagnosis and drug designing of abnormalities of the male genital tract. The use of molecular diagnostic methods is becoming more prevalent in clinical microbiology laboratories. The most popular molecular diagnostics for diagnosing disorders are various nucleic acid amplification formats. Due to its exceptional sensitivity and specificity, PCR is unquestionably the method of choice in many therapeutic settings. Real-time PCR is now replacing many traditional PCR techniques since it provides for quicker quantification and detection of the PCR result as well as the identification of various pathogen strains via melting curve analysis. The capacity to quantify microbe numbers by quantitative polymerase chain reaction (qPCR) has grown in significance as a tool for assessing illness development, prognosis, and therapy efficacy. Microarray is a technology with great potential which can concurrently identify many infections with high sensitivity.[67]

6.6.1 Polymerase Chain Reaction: For the diagnosis of male disorders, polymerase chain reaction (PCR) is commonly used molecular diagnostic tool. Target DNA is extracted and subsequently denatured at high temperatures in principle. It begins with the DNA polymerase enzyme copying the DNA template strand, and then the DNA polymerase enzyme anneals specific oligonucleotides to the DNA. Millions of copies of the same DNA are produced by repeating this process 30–40 times.[68] The Multiplexed-PCR assay offers a significant amount of promise for application in sperm screening programmed for pathogens in clinics that treat infertility and STDs, as well as in sperm banks. The multiplex PCR (M-PCR) assay streamlines the work flow, making it suitable for use in ordinary diagnostic laboratories that are equipped with only the most fundamental molecular facilities. Therefore, this M-PCR test has a significant amount of potential to be utilized in screening programs for sperm pathogens in sperm banks, as well as in clinics that treat infertility and STDs. It is possible that further research utilizing M-PCR in a variety of patient demographics and clinical settings would help shed light on the relationship between STD pathogens and male infertility. In total, seven other sexually transmitted diseases including HPV were examined using both M-PCR and sPCR. The total prevalence of sexually transmitted diseases (STDs) in the sperm of asymptomatic individuals was estimated to amount to 42 specimens out of 52 (55.3%), all of which showed the presence of at least one pathogen.[69]

6.6.2 Line probe assay: In the line probe assay (LiPA), particular oligonucleotide probes are affixed to a nitrocellulose strip in parallel lines and then hybridised with biotin-labeled PCR products at specified sites on the strip.[70]

6.6.3 Microarrays: Glass, silicon, plastic, or nylon membranes are used to create a 2-dimensional matrix of biomolecules for microarray technology. The immobilised biomolecule, such as an oligonucleotide or a protein, can be detected using microarrays, which can evaluate both nucleic acids and antibodies. Target labelling using fluorescent probes or antibodies can be

detected with super advanced scanners, indicating a positive reaction. For example, DNA probes can be immobilised in 3-dimensional gel drops or electrochemically detected without the need for prior PCR amplification. In the future, DNA and serological microarrays are expected to play a significant role in clinical microbiology laboratory technology.[70]

Different illnesses are thought to be caused by extracellular miRNA expression that is abnormal. The prospect of identifying potent miRNA markers in male infertility has been hypothesised since changed expression of miRNAs, along with altered expression of several testis-specific mRNAs, has recently been described in male infertility problems.

6.7 Genetic markers: As genetic identifiers for the differentiation of infections, DNA barcodes have proven to be incredibly beneficial, especially for the quick discovery of novel and developing diseases/pathogens. Genetic markers which are usually DNA sequences that have been mapped to specific chromosome regions. Points of variation in a gene's sequence can be used to identify individuals or species, or to relate an inherited disease to a gene via genetic linkage to neighboring but presumably unknown or uncharacterised genes. An SNP is an example of a single nucleotide polymorphism (SNP).[71]

Men may be at risk for infertility due to common polymorphisms in the genes of pro-inflammatory and anti-inflammatory cytokines, which can change their synthesis and activity in response to infections. In human populations, there are many different DNA sequence variations known as polymorphisms. SNP, or single nucleotide polymorphism, is one of them.[72]

6.8 Protein markers: In any case, novel and additional biomarkers are still needed from the perspective of a patient. Routine clinical tests for determining biomolecule concentrations in bodily fluids for prognosis, diagnosis, and monitoring of certain disorders allow opportunity for improvement in assay performance as well as statistical sensitivity and specificity. The lack of standardization in testing is generally due to an analyte that isn't well-defined.[73]

Since the early days of mass spectrometry-based proteomics, doctors and researchers have worked together to find protein markers. Many possibilities have been identified through exploratory case-control investigations applied to clinical sample cohorts containing plasma, serum and urine samples. Protein markers have made it to the clinic, but the number is still quite small. Initially, this translation lag was attributed to technical inaccuracy and the high complexity of the applied MS-based strategies, but it has since become clear that a small number of samples, invalid specimen inclusion, and a lack of considerate study design and standard procedures have influenced successful biomarker development more than anything else. A strong platform for quantitative biomolecule assessments has been established using MS-technology, which has matured since then. These platforms enable the proteomics discipline to reach a new era of MS applications by offering a bridge among basic research and clinical validation.[74]

1708 proteins were found in the combined seminal plasma of 5 patients with prostatitis, as determined by mass spectrometry. A comparison of this list with a catalog of seminal plasma plasma proteins previously published in the seminal plasma of (pooled) five fertile, healthy controls revealed 1464 proteins entities in common, 413 proteins unique to the healthy controls, and 254 proteins unique to the prostatitis group. Assured list of 59 putative prostatitis biomarkers was created using this data after a set of criteria was applied to it. Of these, 33 were massively increased in prostatitis compared to control and 26 were decreased.[75]

Proteins, among the various forms of biomarkers, can be extremely sensitive, allowing them to be identified in a very small amount of a sample to diagnose a specific type of disease in its early stages. The changes in the molecular signature of protein during the course of disease, the screening of the proteins in the tissues and different body fluids serves as indicators for the pathological conditions.[76]

Proteins are postgenomic effectors of cellular and molecular events, protein expression profiling, or the comprehensive identification and quantification of proteins present in a fluid or a cell population, has become an important and established tool in understanding the mechanics of biological systems. The genetic foundation of human disease is far more complicated than previously imagined, with a plethora of metabolic and regulatory pathways, post-translational changes, and complex protein-protein interactions all playing a role. As a result, organ- and tissue-specific protein profiling studies will immediately contribute to a better understanding of the molecular signature of the pathological conditions and hence contributing to designing of better therapeutic drugs.[77]

Protein profiling has greatly contributed in the selection of distinctive proteins based on their response to the particular conditions, either they are upregulated or downregulated or choosing the differentially expressed proteins and using them for the designing of new therapeutic drugs and reliable diagnosis. The defined and known group of proteins could be used for the identification of the unknown group of proteins and hence, making proteins suitable for the clinical diagnostics.[78]

REFERENCES

1. Bieniek JM, Lo KC. Imaging in Male Reproductive Medicine. Encyclopedia of Reproduction, 2018; 217–24.
2. Brock G, Hsu GL, Nunes L, Von Heyden B, Lue TF. The anatomy of the tunica albuginea in the normal penis and Peyronie's disease. The Journal of Urology, 1997; 157(1): 276-81.
3. Sam P, LaGrange CA. Anatomy, Abdomen and Pelvis, Penis. In StatPearls. StatPearls Publishing: 2018.
4. Setchell B, Breed W. Anatomy, Vasculature, and Innervation of the Male Reproductive Tract. Knobil and Neill's Physiology of Reproduction, 2006; 771–825.
5. Creasy DM, Chapin RE. Male reproductive system. Haschek and Rousseaux's handbook of Toxicologic Pathology: 2013; 2493-598.
6. Rodriguez CM, Kirby JL, Hinton BT. The development of the epididymis. In The Epididymis: From Molecules to Clinical Practice. Springer, Boston, MA: 2002; 251-267.
7. Robaire B, Hermo L, Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. The Physiology of Reproduction, 1988; 1: 999-1080.
8. Chapple C. Tissue engineering of the urethra: where are we in 2019. World Journal of Urology, 2020; 38(9): 2101-05.
9. Aumuller G, Riva A. Morphology and functions of the human seminal vesicle. Andrologia, 1992; 24(4): 183-96.
10. Lee JY, Dada R, Sabanegh E, Carpi A. Agarwal A Role of genetics in azoospermia. Urology, 2011; 77(3): 598-601.
11. Roberts KP, Pryor JL. Anatomy and physiology of the male reproductive system. In Male Infertility and Sexual Dysfunction. Springer, New York, NY: 1997;1-21
12. Gurung P, Yetiskul E, Jialal I. Physiology, male reproductive system. In StatPearls. StatPearls Publishing: 2021.
13. Yue B, Cui Z, Kang W, Wang H, Xiang Y, Huang Z, Jin X. Abdominal cocoon with bilateral cryptorchidism and seminoma in the right testis: a case report and review of literature. BioMed Central Surgery: 2019; 19(1): 1-5.
14. Toliczenko-Bernatowicz D, Matuszczak E, Komarowska M, Hermanowicz A, Dębek W. Epidemiology, pathophysiology, and pathogenesis of cryptorchidism. Evaluation and treatment of undescended testicle. PediatriaPolska-Polish Journal of Paediatrics, 2020; 95(1): 37-43.
15. Hutson JM, Balic A, Nation T, Southwell B. Cryptorchidism. In Seminars in Pediatric Surgery. WB Saunders, 2010; 19(3): 215-24.
16. Kaushik P, Kaushik G An assessment of structure and toxicity correlation in organochlorine pesticides. Journal of Hazardous Materials, 2007; 143(1-2): 102-11.
17. Virtanen HE, Cortes D, RajpertDe Meyts E, Ritzén EM, Nordenskjöld A, Skakkebaek NE, Toppari J. Development and descent of the testis in relation to cryptorchidism. ActaPaediatrica, 2007; 96(5): 622-7.
18. Rajpert-De Meyts E, Main KM, Toppari J, Skakkebaek NE Testicular dysgenesis syndrome, cryptorchidism, hypospadias, and testicular tumors. Endocrinology: Adult and Pediatric. WB Saunders: 2010; 7(2): 2499-513.
19. Mathers MJ, Sperling H, Rubben H, Roth S. The undescended testis: diagnosis, treatment and long-term consequences. Deutsches Arzteblatt International, 2009; 106(33): 527–32.
20. Cortes D, Thorup JM, Visfeldt J. Cryptorchidism: aspects of fertility and neoplasms. Hormone Research in Paediatrics, 2001; 55(1): 21-27.
21. Nichols CR. Testicular cancer. Current problems in Cancer, 1998; 22(4): 187-274.
22. Smith ZL, Werntz RP, Eggener SE. Testicular cancer: epidemiology, diagnosis, and management. Medical Clinics, 2018; 102(2): 251-264.
23. Bahrami A, Ro JY, Ayala AG. An overview of testicular germ cell tumors. Archives of pathology & laboratory medicine, 2007; 131(8): 1267-1280.
24. Nauman M, Leslie S. Nonseminomatous Testicular Tumors. StatPearls 2022.
25. Cheng L, Albers P, Berney DM, Feldman DR, Daugaard G, Gilligan T, Looijenga LH Testicular cancer. Nature Reviews Disease Primers, 2018; 4(1): 1-24.
26. Mazhar D, Williams M. Testis Cancer. In The Scientific Basis of Urology 3, 2010; 403-14. CRC Press.
27. Garner MJ, Turner MC, Ghadirian P, Krewski D. Epidemiology of testicular cancer: an overview. International Journal of Cancer, 2005; 116(3): 331-9.
28. Swerdlow AJ. Testicular cancer: epidemiology and molecular endocrinology. Hormones, Genes, and Cancer, 2003; 413.
29. Tai Lahans LAC. Integrating conventional and Chinese medicine in cancer care: A clinical guide. Elsevier Health Sciences, 2007.
30. Pienta KJ, Esper PS. Risk factors for prostate cancer. Annals Of Internal Medicine, 1993; 118(10): 793-803.
31. Dunn MW, Kazer MW. Prostate cancer overview. In Seminars In Oncology Nursing. WB Saunders, 2011; 27(4): 241-50.
32. Chillon M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, Estivill X. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. New England Journal of Medicine, 1995; 332(22): 1475-80.
33. Chiba K, Enatsu N, Fujisawa M. Management of non-obstructive azoospermia. Reproductive Medicine and Biology, 2016; 15(3): 165-73.
34. Cocuzza M, Alvarenga C, Pagani R. The

epidemiology and etiology of azoospermia. Clinics, 2013; 68: 15-26.
35. Lee C H, Akin-Olugbade O, Kirschenbaum A. Overview of prostate anatomy, histology, and pathology. Endocrinology and Metabolism Clinics, 2011; 40(3): 565-75.
36. Sharma M, Leslie S. Azoospermia. In StatPearls. StatPearls Publishing: 2022.
37. Sihag P, Tandon A, Pal R, Jain B K, Bhatt S, Kaur S, Sinha A. Sonography in male infertility: a look beyond the obvious. Journal of Ultrasound, 2018; 21(3): 265-76.
38. Alsaikhan B, Alrabeeah K, Delouya G, Zini A. Epidemiology of varicocele. Asian Journal of Andrology, 2016; 18(2): 179.
39. Fretz PC, Sandlow JI. Varicocele: current concepts in pathophysiology, diagnosis, and treatment. Urologic Clinics, 2002; 29(4): 921-37.
40. Rigano E, Santoro G, Impellizzeri P, Antonuccio P, Fugazzotto D, Bitto L, Romeo C. Varicocele and sport in the adolescent age. Preliminary report on the effects of physical training. Journal of Endocrinological Investigation, 2004; 27(2): 130-32.
41. Cho CL, Esteves SC, Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian Journal of Andrology, 2016; 18(2): 186–93.
42. Sakamoto Y, Ishikawa T, Kondo Y, Yamaguchi K, Fujisawa M. The assessment of oxidative stress in infertile patients with varicocele. The British Journal of Urology, 2008; 12: 1547-52.
43. Hassanin AM, Ahmed HH, Kaddah AN. A global view of the pathophysiology of varicocele. Andrology, 2018; 6(5): 654-61.
44. Leslie SW, Siref LE, Khan MA. Male infertility. In StatPearls. StatPearls Publishing: 2020.
45. Sandean DP, Lotfollahzadeh S. Peyronie Disease. In StatPearls. StatPearls Publishing: 2020.
46. Rupp TJ, Leslie SW. Epididymitis. In StatPearls. StatPearls Publishing: 2017.
47. Taylor SN. Epididymitis. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America, 2015; 61(8): S770–73.
48. Dalpiaz A, Schwamb R, Miao Y, Gonka J, Walzter W, Khan SA. Urological Manifestations of Henoch-Schonlein Purpura: A Review. Current Urology, 2015; 8(2): 66–73.
49. Nickel JC. Chronic epididymitis: a practical approach to understanding and managing a difficult urologic enigma. Reviews in Urology, 2003; 5(4): 209–15.
50. Cek M, Sturdza L, Pilatz A. Acute and chronic epididymitis. European Urology Supplements, 2017; 16(4): 124-31.
51. Man J, Cao L, Dong Z, Tian J, Wang Z, Yang L. Diagnosis and treatment of epididymal tuberculosis: a review of 47 cases. The Journal of Life & Environmental Sciences, 2020; 8: e8291.
52. Buttaravoli P, Leffler SM (Eds.). Urologic Emergencies. Minor emergencies. Elsevier Health Sciences, 2012; 301-05.
53. Schuppe HC, Meinhardt A, Allam JP, Bergmann M, Weidner W, Haidl G. (2008). Chronic orchitis: a neglected cause of male infertility. Andrologia, 2008; 40(2): 84-91.
54. Azmat CE, Vaitla P. Orchitis. In StatPearls. StatPearls Publishing; 2020.
55. Wu H, Wang F, Tang D, Han, D. Mumps orchitis: clinical aspects and mechanisms. Frontiers in Immunology, 2021; 12: 582946.
56. Bellini WJ, Icenogle JP, Hickman CJ. Measles, Mumps, and Rubella Viruses. Clinical Virology Manual, 2016; 293-310.
57. Masarani M, Wazait H, Dinneen M. Mumps orchitis. Journal of the Royal Society of Medicine, 2016; 99(11): 573-75.
58. Sikka SC, Hellstrom WJ. Current updates on laboratory techniques for the diagnosis of male reproductive failure. Asian Journal of Andrology, 2016; 18(3): 392.
59. Drabovich AP, Saraon P, Jarvi K, Diamandis EP. Seminal plasma as a diagnostic fluid for male reproductive system disorders. Nature Reviews Urology, 2014; 11(5): 278-88.
60. Shokeir MHK, Lefebvre Y. Diagnosis of Hypothalamic-Pituitary Disorders in Andrology. In Diagnosis in Andrology. Springer, Dordrecht, 1980; 5-18.
61. Sokol RZ. Endocrine evaluation in the assessment of male reproductive hazards. Reproductive Toxicology, 1988; 2(3-4): 217-22.
62. Mittal PK, Little B, Harri PA, Miller FH, Alexander LF, Kalb B, Moreno CC. Role of imaging in the evaluation of male infertility. Radiographics, 2017; 37(3): 837-54.
63. Christensen JD, Dogra, VS. The undescended testis. In Seminars In Ultrasound, CT and MRI. WB Saunders, 2007; 28(4): 307-316.
64. Lee J, Binsaleh S, Lo K, Jarvi K. Varicoceles: the diagnostic dilemma. Journal of Andrology, 2008; 29(2): 143-146.
65. Damodaran, T. V. Genotoxicities and infertility. In Reproductive and Developmental Toxicology. Academic Press: 2011; 923-47.
66. Ferlin A, Calogero AE, Krausz C, Lombardo F, Paoli D, Rago R, Scarica C, Simoni M, Foresta C, Rochira V, Sbardella E, Francavilla S, Corona G. Management of male factor infertility: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS): Endorsing Organization: Italian Society of Embryology, Reproduction, and Research (SIERR). Journal of Endocrinological Investigation: 2022; 45(5): 1085–113.
67. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, Yao JD, Wengenack NL, Rosenblatt JE, Cockerill FR, Smith TF. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clinical Microbiology Reviews, 2006; 19(1): 165–256.

68. Wright PA, Wynford TD. The polymerase chain reaction: miracle or mirage. A critical review of its uses and limitations in diagnosis and research. The Journal of Pathology, 1990; 162(2): 99-117.
69. Gimenes F, Souza RP, Bento JC, Teixeira JJ, Maria-Engler SS, Bonini MG, Consolaro ME. Male infertility: a public health issue caused by sexually transmitted pathogens. Nature Reviews Urology, 2014; 11(12): 672-87.
70. Kurkela S, Brown DW. Molecular diagnostic techniques. Medicine, 2009; 37(10), 535-40.
71. Abu-Salah KM, Zourob MM, Mouffouk F, Alrokayan SA, Alaamery MA, Ansari AA. DNA-Based Nanobiosensors as an Emerging Platform for Detection of Disease. Sensors, Basel, Switzerland: 2015; 15(6): 14539–68.
72. Aziziaram Z. C3953T genetic variation in interleukin 1β and idiopathic male infertility: a systematic review and meta-analysis. Central Asian Journal of Medical and Pharmaceutical Sciences Innovation, 2021; 1(6): 242-249.
73. Mayeux R. Biomarkers: potential uses and limitations. The journal of the American Society for Experimental Neuro Therapeutics, 2004; 1(2): 182-8.
74. Hawkridge AM, Muddiman DC. Mass spectrometry–based biomarker discovery: toward a global proteome index of individuality. Annual Review of Analytical Chemistry (Palo Alto, Calif.), 2009; 2: 265.
75. Kagedan D, Lecker I, Batruch I, Smith C, Kaploun I, Lo K, Jarvi KA. Characterization of the seminal plasma proteome in men with prostatitis by mass spectrometry. Clinical Proteomics, 2012; 9(1): 1-20.
76. Alharbi RA. Proteomics approach and techniques in identification of reliable biomarkers for diseases. Saudi Journal of Biological Sciences, 2020; 27(3): 968–74.
77. Cho WC. Proteomics technologies and challenges. Genomics, Proteomics & Bioinformatics, 2007; 5(2): 77–85.
78. Emmanouilidi A, Fyffe CA, Ferro R, Edling CE, Capone E, Sestito S, Rapposelli S, Lattanzio R, Iacobelli S, Sala G, Maffucci T, Falasca M. Preclinical validation of 3-phosphoinositide-dependent protein kinase 1 inhibition in pancreatic cancer. Journal Of Experimental & Clinical Cancer Research: CR, 2019; 38(1): 191.