CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

To Investigate the Ubiquitous Risk Factor Associated with Bladder Cancer in Twin Cities of Pakistan (Rawalpindi and Islamabad)

by

Tehmina Hashim

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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Dedicated to

My Most loving Brother

Muhammad Danish Malik

Whose prayers took me out from any trouble

My Beloved Family

Whose encouragement, Prayers & support are always for me & Malik

Muhammad Qasim

CERTIFICATE OF APPROVAL

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"I dedicated my thesis to my loving brother **Muhammad Danish Malik"**

Tehmina Hashim

Abstract

Bladder cancer is seventh leading cause of cancer death among men worldwide. Pakistan faces a double burden of disease with a significant incidence of cancers and a rising trend in risk factors like other developing countries. This study included data of 50 individuals of bladder cancer from 3 different hospitals. Epidemiological data was recorded by standard questionnaire and different risk factors which are associated with bladder cancer were observed. Simple descriptive statistics was applied to investigate the most prevalent factor of bladder cancer, association between these factors were obtained with the help of chi square. Logistic and regression model was also applied. Bladder cancer was most prevalent in males as compared to females. Family history, smoking, surgery of lower urinary tract and exposure with arsenic were the major contributing factors of bladder cancer. Passive smokers, on the other hand, have an equivalent chance of developing bladder cancer. Incidence of bladder cancer was not associated with patients occupation accept the person who had exposure with arsenic, amines and with certain pesticides. This study suggested that family history of the disease, smoking, time and depth of smoking, metastasis, grade, anemia and diabetes are most leading factors in the development of bladder cancer. By using statistical analysis that there was statistically insignificant association between residential areas and bladder cancer (p=0.868). There is non-significant association between residential areas and bladder cancer (p> .05). The value for abdominal surgery (1) = 1.082, with a 95 % confidence interval of (.334, 3.501), indicates that patients who have had abdominal surgery have a 1.082 times higher risk of bladder cancer than those who have not had abdominal surgery. This suggests that abdominal surgery may have a role in bladder cancer progression. To conclude, smoking over an incredibly long time, previous abdominal surgeries, and gender are all predicted to be significant risk factors for bladder cancer.

Contents

List of Figures

List of Tables

Abbreviations

BC	Bladder cancer
BCG	Bacillus Calmette- Guerin
CIS	Carcinoma in situ
HMS	Hospital Management System
MRI	Magnetic resonance imaging
NAT2	N Acyl transferase 2
NIMBC	Non muscle invasive bladder cancer
TURBT	Transurethral resection of bladder tumor
UCB	Urothelial carcinoma of Bladder
UCC	Urothelial cell carcinoma
UTI	Urinary tract infection

Chapter 1

Introduction

1.1 History

According to the most recent GLOBOCAN data, bladder tumor accounts for three percent among all cancer diagnoses worldwide, with developed world having the highest prevalence, In United States bladder cancer is 6th most common type and 90 % of bladder tumor diagnoses are higher in 55 and older, and males are four times more probable to develop the disease [1]. Cancers are caused by aberrant gene function and altered gene expression patterns, growing evidence suggests that acquired epigenetic abnormalities interact in collaboration with genetic mutations to promote this deregulation [2]. Recent advances in immunotherapy, biomarkers, and advanced imaging have all become progressively effective for the bladder cancer diagnosis, which has been more common as in last 2 decades, and this issue will discuss recent developments when it comes to bladder cancer treatment.

Several prognostic factors can be linked to bladder cancer's evolutionary recurrence. Whereas the permeability through into lamina propria (stage), the development of invasive carcinoma, and the presence of invasive carcinoma are all aspects to be considered. Although these factors identified are well-known, the biochemical foundation for the illness subgroups within each of these causative agents is less well understood [3]. More than 90 percent of invasive lobular cancers occur in the renal tract, with accounting for 8% and the ureter and urethra accounting for the remaining 2% and the most commonly histologic subtype in the

United States is urothelial (transitional cell) carcinoma, which can occur everywhere. The ureter bladder and proximal two-thirds of the urethra all seem to have transitional epithelium [4]. In the United States, tumor of bladder is the biggest cause of mortality and morbidity, that is three times more likely in males than in women.

Bladder cancer will cause approximately 16,000 deaths (11,510 males and 4490 women) across the same time period [5]. One of the most common malignancy found in humans is bladder cancer develops in two ways have two stages of developmen: papillary and nonpapillary, which correlate to clinically distinct forms of the cancer. The majority of bladder cancers are caused by chemicals, with tobacco smoking being the leading cause. Bladder neoplasms can arise in any layer of the bladder. They are either epithelial or nonepithelial (mesenchymal), with epithelial cells accounting for over 95 percent, Urothelial malignancies are epithelial malignancies that have differentiated into normal urothelial and transitional carcinoma has just been replaced by urothelial carcinoma [6].

1.2 Cancer

Multiple alterations in gene expression lead to association with alterations and balance of cell proliferation and cell death, eventually transforming into a swarm of invasive cells organs and metastasis to distant sites, causing considerable morbidity and if remain death of the host, cancerous tissue through the circulation or lymphatics toward other internal organs when they escape the clutches from a tumor. Cancer can develop everywhere across the body and is designated that after organ where it first emerged. For instance, even though breast cancer originates in the breast then spreads (metastasizes) to other parts of the body but it is still called breast cancer. Cancer is a general term applied to a variety of diseases characterized by uncontrollable cell division that can invade and destroy normal human tissue. Cancer cells have potential to spread out to other organs in your body, while cancer is world's second most common cause of death [7].

1.2.1 Bladder Cancer

The most frequent tumor is bladder tumor that requires urological consultation and surgery on a consistent basis, etiology of malignancy of bladder is unclear, with the exception of smoking and some employment risks. Despite the fact that the majority of patients with bladder cancer do not have a family history of urinary tract transitional cell carcinoma. While pathologists have traditionally relied on factors such as histologic tumor type, grade, depth of invasion, and the presence or absence of vascular invasion. To enhance our ability to sub classify these patients, we've recently included innovative techniques, including as flow cytometer, monoclonal antibodies, proliferative rate measurement, and cytogenetic and molecular genetics [8].

Urothelial bladder cancer (UBC) is the world's seventh most common cancer in males and the seventeenth most common cancer in women. In the Western world, in developed countries UBC is more common, in case of females bladder cancer is fourth and sixth most common and this frequency combined with the fact that UBC is relapsing, means that UBC imposes a major burden on health-care systems [9].

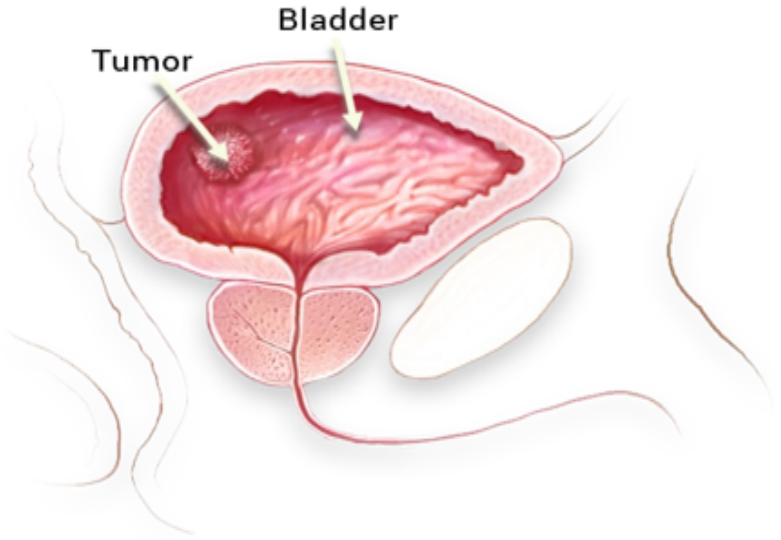


FIGURE 1.1: Bladder Tumor[10]

1.3 Worldwide Incidence of Bladder Cancer

According to GLOBOCAN estimates, in 2012, there have been 14.1 million new cancer cases worldwide and 8.2 million deaths. The implication of cancer has changed over time, with undeveloped countries now responsible with around 57 % and 65% of cancer deaths worldwide. Despite the reality that male and female cancer incidence rates are around twice as high in developed countries as in underdeveloped countries, mortality rates are only 8% to 15% higher in developed countries [11].

From 2009 to 2013, there was a 26-fold difference in bladder and other gallstone cancer mortality rates among women, ranging from 0.8 deaths per 100,000 in South Africa to 21.2 deaths per 100,000 in Chile. Men's incidence rates ranged from 0.6 per 100,000 in the United Kingdom and Ireland to 9.9 per 100,000 in Chile. The mortality rates for women were greater [12]. Since 1993, men's cancer death rates have decreased by 1.5 percent per year, whereas women's cancer death rates have decreased by 0.8 percent per year since 1992. The incidence rates for the three most prevalent cancer sites in males (lung and bronchus, colon and rectum, and prostate) and breast, colon, and rectum cancers in women have similarly decreased and in African and American men and women have % and 18% higher death rates from all cancers combined than White men and women, according to race and ethnicity analyses [13].

1.4 Bladder Cancer Properties

Bladder cancer generally begins in the cells that are present at the lining of the bladder (urothelial cells), kidneys and the tubes (ureters) that connect the kidneys to the bladder also contains urothelial cells, urothelial carcinoma can arise in the kidneys and ureters and it is even more prevalent in the bladder and cell malfunction characterizes cancer cell metastasis, which is a step wise process. Some of the major pathological characteristics of cancer cells comprise their mechanical properties, as well as their tendency to infiltrate into surrounding tissues, transmigrate,

and proliferate in different locations. The transformation from non-metastatic cell to metastatic cell is a crucial step in cancer, and it is still unclear how it occurs.

However, there is evidence that the actin cytoskeleton is altered and rearranged throughout cancer progression [14]. The cytoskeleton microstructure modified when cancer can spread, adhere, multiply, and divide is primarily designated as abnormal structural properties of cells. Several studies of different ailment using experimental techniques have revealed that abnormalities are linked to cell mechanical properties, particularly in cancer metastasis. In reality some research suggests that cancer cells are stiffer than benign cells, while others show that cancer is characterized by a decrease in cell stiffness, i.e. metastatic cancer cells have an elastic molecular structure[15].

To detect or diagnose cancer, doctors utilize an assortment of tests, to examine if the cancer cells spread to other regions of the body from where it originated are also advised. When this happens, it's known as metastasis. MRI scan can tell whether the malignancy has spread, for example, imaging examinations can create images of the inside of the body in most types of cancer, a biopsy is the only guaranteed technique for a doctor to identify whether a body part contains cancer. Local probes, in which a section of the cell is deformed, whole-cell mechanical loading, and the mechanical stressing of a population of cells are three types of experimental techniques that have been developed over the years to examine the mechanical characteristics of cells.

Mechanical stresses can be detected by living cells and converted into biological responses, Biological and metabolic signals are also known to affect cells ability to detect, create, and bear mechanical stresses. Research into the mechanics of single cells, subcellular components, and biological molecules has exploded in the latest decade, with considerable implications [16]. Cholesterol, as one of the major lipid components of plasma membrane in all mammalian cells, has a substantial influence on endothelial cell mechanical properties and certain abnormalities in blood lead to various kinds of cancers, In addition, the viscoelasticity fluctuation of cell membranes under the cholesterol effect was tracked simultaneous it revealed that cholesterol repletion increased the Young's modulus and the complex modulus

of the EA.hy926 cell by over 30%, respectively, and that the amplitudes of both the elasticity and viscosity oscillations at a peaked level [17].

Cancer cells can deform more easily than healthy cells, and extremely aggressive cancer cells can deform more easily than less invasive cancer cells. According to several studies6–12, and it has been claimed that cancer cells elastic features play a crucial part in the metastatic process. This makes it certain the mechanism of metastasis, which involves cells invading neighboring cells while huge numbers of bladder cancers are diagnosed early, when they are highly curable. Even early-stage bladder cancers, however, might recur after treatment. As a result, individuals with bladder cancer may require follow-up testing for years following treatment to check for recurrence [18].

In our search for new markers of bladder cancer development, we previously reported that FGFR2-IIIb (fibroblast growth factor receptor 2-IIIb) was expressed in normal bladder urothelial cells and that its expression was decreased in a subset of bladder carcinomas, with this change of expression being associated with a poor prognosis. The endocrine system in mammals is governed by four genes (FGFR1, FGFR2, FGFR3, and FGFR4) [19]. GFRs are glycoproteins that are made up of two or three extracellular immunoglobulin (IG) like domains, a hydrophobic trans membrane region, and a cytoplasmic component with a tyrosine kinase catalytic site. Various receptor isoforms are produced by alternative mRNA splicing methods, while FGFR2-IIIb and FGFR2-IIIc are produced by a mutually exclusive splicing event in which the second part of the juxta membrane is excised [20]. Cancer stem-like cells are a tiny segment of cells inside a tumor which have stem-like characteristics like self-renewal and the ability to differentiate into new cells. Many researchers suggest these tiny cells are involved in cancer start, development, metastasis, and that they are associated with increased chemo- and radio-resistance and mH2A may operate as a tumor suppressor in a variety of human malignancies, according to recent research. The expression of mH2A has been found to be highly down regulated in many other types of tumor, including the bladder, lung, breast, and that the loss of mH2A can accelerate cancer progression and the metastatic capability of melanoma and cells of bladder tumors [21].

1.5 Bladder Cancer Incidence in Pakistan

No cancer incidence data from Pakistan has been released in the five decades of independence. Incidence data for the Karachi South district (1.7 million people) from 1995 to 1997 are shown below. During this time, there were a total of 4,268 new cancer cases, with 2,160 cases in men and 2,108 cases in women. 95.3 % of the incident cases were found to be microscopically verified. For any demographic in Pakistan, cancer incidence data has never been available. Only occasional relative frequency information from hospitals, such as Karachi's Jinnah Postgraduate Medical Center (JPMC), and a network of hospital registries managed by the Pakistan Medical and Research Council, are accessible and several decisions affect the hospital-based information [22].

In many Asian countries, cancer is becoming a severe health issue, and it has surpassed heart disease as the top cause of death in the region. In Asia, there were more than 3 million new cancer cases and more than 2 million cancer deaths in 2000, and forecasts showing the number of new cancer cases will increase to 7.1 million by 2020 if current prevention and care strategies are not modified[23].With the help of ACSP, KCR calculated the incidence of Karachi Division (1998-2002) population of 9,802,134 [52 percent males (M) and 47 percent females (F)] annual growth rate 3.52 and Quetta Division (1998-1999) population of 759,245 [56 percent males (M) and 44 percent females (F)] annual growth rate With a few variations, the cancer profile of Hyderabad resembles that of Karachi from 1995 to 2004. From 1998 to 2002, the age-standardized rates (ASR) for cancer in Hyderabad (all sites) were 91.6/100,000 males and 96.0/ 100,000 females [24].

Males had an age-standardized rate (ASR) of 139.11100000 and females had an age-standardized rate (ASR) of 169.5/100000 from 1995 to 1997 in Kansas. From 1998 to 2002, males had a rate of 179.0/100000, while females had a rate of 204.1/100000. Lung (21.3), oral cavity (14.2), urinary bladder (9.0), and larynx were the most prevalent malignancies in males (ASR per 100,000) in the 1995-1997 data (8.8). Fetal malignancies are tumor that originate in the female reproductive system, Females (1995-1997, CI 48.2; 52.4) Males were 49.5 years old (% confidence

interval 47.5–51.4) and females were 53.7 years old (95 % confidence confidence interval 51.5–55.6) between 1998 and 2002. Histological confirmation persisted at 97 percent with 47.1 percent in grade II or I and 65.0 percent in stages III and IV [25].

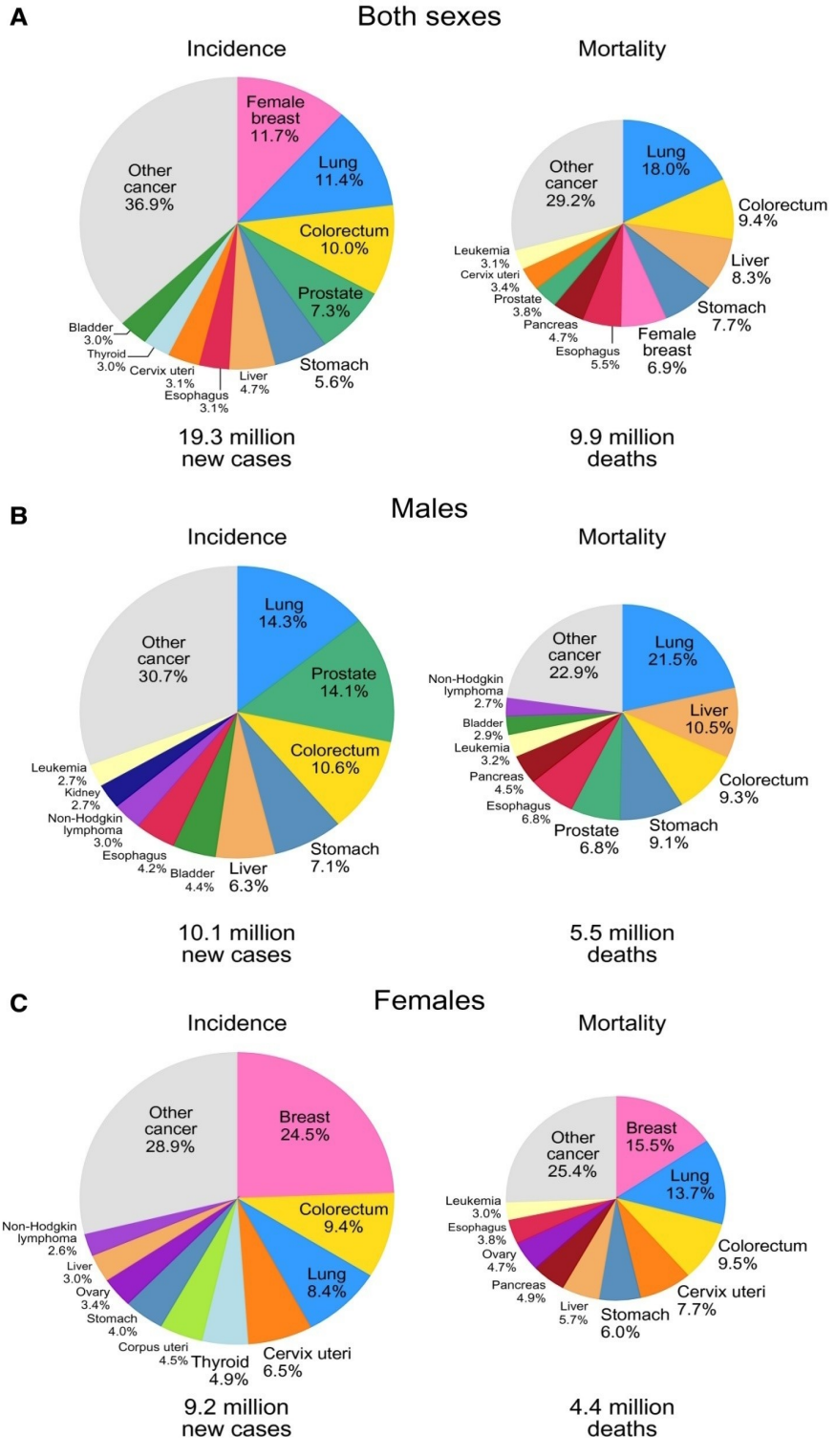


FIGURE 1.2: Incidence of Bladder Cancer in Pakistan [10]

1.6 Problem Statement

Bladder cancer is diagnosed in very early ages in Pakistani population as representation age in Pakistan is 40 or 50 years to investigate the most prevalent epidemiological factors which are associated with bladder cancer in Pakistani population.

1.7 Aims and Objective

Objectives to determine the most common cause of bladder cancer in Pakistani individuals.

- Determine the mean age, height, and weight of Bladder cancer patients.
- To investigate the risk of bladder cancer in relation to cigarette smoking, as well as medical and dietary history over a significant period of time.
- To investigate the relationship between bladder cancer and several epidemiological (residential area and smoking)factors.

Chapter 2

Literature Review

2.1 Types of Bladder Cancer

Cells of urinary bladder grow more rapidly and becomes out of control. Bladder cancer develops additional cancer cells proliferate tumors arises which can move to others area in body over time and in lower pelvis, part of the body with a spherical interior called bladder. Its walls that is elastic and stretchy, while respective organ has ability to stretch to store urine and contract to release it. While the expression of genes evolved in cancer metabolism or tumor cell proliferation is increased in BC metabolism. Glycogen metabolism play crucial role in the progression of bladder cancer [26].

2.2 Urothelial Carcinoma (Transitional Cell Carcinoma)

Cancer in which lining of transitional cells are affected is urothelial carcinoma, develops in the inner lining of bladder. When your bladder is full urothelial cells expand contract when bladder is empty and insides of ureters and urethra are lined with same cells, and malignancies can occur there as well most prevalent type of bladder cancer is urothelial carcinoma [27].

2.2.1 Squamous Cell Carcinoma

In the United States, squamous cell carcinomas represent approximately 1% to 2% of malignant tumors. The cells when examined under a microscope. Cancer of the bladder interferes with the replication of flat cells situated on the skin's surface [28].

2.2.2 Small Cell Crcinoma

Initial phase melanomas account for even less than 1% of urological malignancies. They arise in neuroendocrine cells which are nerve-like cells. These tumors grow quickly and require chemotherapy similar to that used to treat small cell carcinoma of the lung [29].

2.3 Environmental Factors for Bladder Cancer

Dietary habits and use of tobacco represent around 3% of new malignancy cases. It is vigorously connected to lifestyle and diet habits. There are lot of positive measures that can overcome tumor risk by consumption of more vegetables and fruit, lessen the eating of red meat and cigarette smoking. The association of cancer-causing agents with an individual's hereditary and acquired constitution determines vulnerability to induction development of cancer, depends upon how an individual's manages the cancer causing agents [30].

Cigarette smoke is the most significant predictor for tumors. Among all the important environmental factors associated with bladder cancer. Tobacco smoke is thought to be directly responsible for 50% of TCCs. Tobacco smoke contains aryl amines and more than 60 additional carcinogens that have been associated to the synthesis of DNA adducts and changes in tumor suppressor expression [31]. The most significant predictor for bladder cancer is occupational exposure to

aromatic amines (2-naphthylamine, 4-aminobiphenyl, and Benzedrine) and 4,4'-methylenebis(2-chloroaniline), that are found in the chemical, dye, and rubber industries, as well as hair dyes, paints and fungicides [32].

2.4 Tumor Staging

The TNM system, which stands for tumors, node, and metastasis, is another criteria that your doctor would most likely examine to establish your overall cancer stage. Each of them will be measured and assigned a number or a "X" if the measurement cannot be determined. Each type of cancer has its own set of symbols, but here's what they represent in general:

Tumor (T): The letters "T" represent between 0 and 4 indicate tumor dimensions and, in some conditions, its position. T0 indicates that no tumor is detectable. The tumor will be worse if the number is greater.

Node (N): Alphabet "N" represent the stage between 0 to 3 indicates while the tumor has progressed to your lymph nodes. Viruses or bacteria are filtered by these glands before they can infect other parts of your body. N0 implies that no lymph nodes are involved. A greater number implies that the cancer is spreading to more lymph nodes, which are located further away from the initial tumor.

Metastasis (M): The letter "M" is preceded by either 0 or 1, indicate that progression of cancer other organs and tissues in your body. A 0 represents that it hasn't, while a 1 implies that it has[33, 34].

The majority of malignancies that involve a tumor are divided into five stages.

There is no cancer in stage 0; only aberrant cells with the potential to develop cancer are present, cancer in situ seems to be another term for it. Stage I cancer refers to tumors are minor and harm only specific region, also known as cancer in its initial phase. Cancer progression in lymph nodes and surrounding tissues are represented in stage II and III.

In Stage IV cancer indicates that it has progression to other organs of body, It's also known as metastatic cancer or advanced cancer[35].

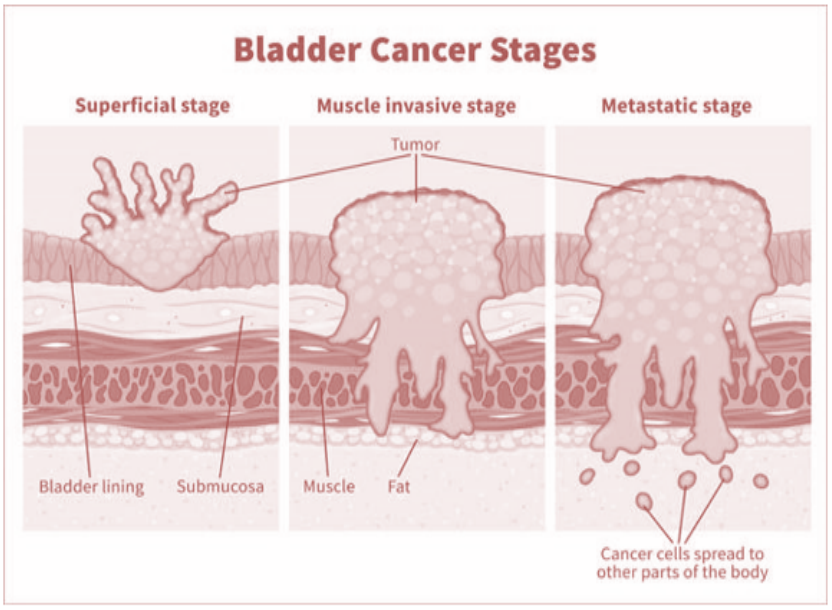


FIGURE 2.1: Stages of Bladder Tumor [36]

2.5 Symptoms of Bladder Cancer

Painless hematuria is one of the presenting sign of all bladder cancers, diagnosis is made by urine cytology and transurethral tumor excision [37]. Bladder cancer symptoms that infuriate people include: Urinary incontinence, urge incontinence, urgency, and dysuria are all symptoms of urinary incontinence. Bladder cancer symptoms that are obstructive include: Reduced stream force, sensation of Anorexia, cachexia, or pallor, lower extremities, edema, and renal failure are several other indicators of metastases or advanced illness [38]. Irritating indicators including frequency and urgency could be the first signs of bladder cancer. According to the Mayo Clinic's CIS experience, 80% had irrigative symptoms. Bladder cancer can induce urinary abnormalities such as the need to urinate more frequently than usually or the inability to urinate at all. During urinating there may be some discomfort or burning. Having difficulty urinating or a weak urine stream, and waking up frequently throughout the night to urinate, are the most

common causes (in men) [39]. A symptom of bladder cancer that has spread to the final stages includes pelvic pain, bone pain, unexpected weight loss, leg inflammation, blood in urine, weariness, and bone tenderness, all of which can indicate more advanced stage disease [40].

In females bladder cancer symptoms include: urine with blood (hematuria), is one of the confirmed indicator of bladder cancer, and it is frequently common indicator of bladder cancer because it's usually painless and might go weeks or even months between occurrences, it's easy for women to overlook, associating with menstruation or menopause. Symptoms of a UTI Many of the symptoms of bladder cancer are identical to those of a urinary tract infection (UTI). Urinary incontinence, pain with urinating, or urinary incontinence may be reported among patients. Discomfort can occur in the flank area, abdomen, or pelvis, and patients may also experience pain in their bones if the cancer has spread to their bones. The loss of appetite is a frequent cancer symptom, and bladder cancer is no unusual, feel fatigued and weak if cancer has progressed or spread. Uterine bleeding after menopause, any blood or spotting observe after menopause could be a sign of bladder cancer or another underlying problem, according to new research on UCB molecular subtypes an oral Lactobacillus preparation is seen to protect from bladder tumor recurrence in a previous clinical investigation. However, limited data has supported this theory to date, and large-scale studies, are needed to see if various microbiological concentrations are connected to UCB progression [41].

2.6 Incidence of Bladder Cancer in Different Cities of Pakistan

According to the IARC, in 2012 new cancers reported globally are 14.1 with the majority of cases (almost 8 million) reported from economically developing countries, which represent for 82 % of the world's population. After prostate cancer, malignancy of bladder is the second more prevalent genitourinary tract tumor worldwide, however in Iran genitourinary tumor are more prevalent, third most common cancer in men [42]. Sindh's live primarily in the south and southwest,

whereas Muhajirs (those who moved from Muslim-majority districts of India during India's partition in 1947) live generally in the lower south while Bulloch reside generally in the southwest, whereas the Pashtuns live mostly in the north however, a huge proportion of Pashtuns live in the southern region. Punjabis also inhabit the south and southeast in significant numbers. In addition, there are a few minor ethnic groups in the upper north, such as the Kashmiris and the Baltics because all of these ethnic groups are very diverse and heterogeneous, cancer statistics obtained from different regions of the country (populated by particular ethnic groups) can differ wildly [43].

2.7 Risk Factor for Bladder Cancer

Tobacco smoking, Age, Gender, family history, diet, medical history, Urinary schistosomiasis, continuously exposed to aromatic amines, total fluid intake, urinary tract infections, some pharmaceuticals, previous cancer or surgery, and genetics) are common risk factors for bladder cancer.

2.7.1 Tobacco Smoking

Most frequent cause of bladder cancer is Cigarette smoking, whereas persons using other tobacco products such as cigars and pipes have higher risk of developing bladder cancer. Smokers have double chance to develop bladder tumor in comparison with nonsmokers, Bladder cancer is reported to be caused by cigarette smoking. A lot of epidemiologic studies have looked into the link between bladder cancer and cigarette smoking, the incidence was enhanced by the quantity of cigarettes and the couple of years consumed smoking [44].

Cigarette smoking is one of major cause of developing bladder when smoking histories are comparable, it has been suggested that In comparison to male smokers, female smokers have a higher chances of bladder cancer. However, further quantification of the issue is essential and higher risk of the disease has been linked to occupational exposure to aromatic amines and other pollutants because most

metabolites are eliminated through the urinary bladder, studies focusing on women revealed increased risks for chemical, metal, tobacco, and agricultural workers. Intake of most chemicals, as well as many foods and nutrients, could be related to the cancer [45].

In both men and women, cigarette smoking is the most well-established risk factor for bladder cancer. While bladder cancer rates have stayed constant over the last three decades, cigarette smoking has decreased significantly in the United States during the same time period. In prior studies, risk estimates for current smokers were typically around three. Yet, over last 50 years, the composition of cigarettes has changed, resulting in a decrease in tar and nicotine concentrations in cigarette smoke8, as well as an apparent increase in the concentration of specific carcinogens such as –naphthylamine, a known bladder carcinogen, and tobacco-specific nitrosamines.

Stopping smoking minimizes the chance of bladder cancer by 30% in the first year, 40% in the past 4 decades, and 60% in the succeeding 25 years. Unfiltered cigarette smokers are 35–50 % more susceptible than filtered cigarette smokers to get bladder cancer. Smoking 'black tobacco' has a number of health risks.[46]. According to previous studies, smoking is a risk factor for 20% to 30% of bladder cancer incidences in women. Most of the earlier studies, on the other hand, were conducted at times or in places where women smoked substantially less. In the last few decades, the content of cigarettes has also varied. While the levels of tar and nicotine have reduced, the levels of other cancer-causing constituents have increased, About half of female bladder cancer cases are caused by smoking, which is similar to the proportion found in males in this and earlier research. The higher frequency of smoking among women is likely to explain the higher proportion of smoking-related tumor of bladder cases among women. This study, researchers discovered a stronger link between smoking and bladder cancer than previously thought. Former smokers were twice as likely as nonsmokers to get bladder cancer, and current smokers were four times more probable. Smoking cessation was linked to a lower risk of bladder cancer, as it found with many other smoking-related cancers [47].

2.7.2 Age

Age is significant indicator of developing bladder malignancy, while tumor of bladder can impact regardless of any era; People in their 40s and 50s are more prone to developing BC, with median age of 70 at the time of diagnosis. [48]. Malignancy of bladder is commonly associated with the age of individuals, due to association among ageing and tumors, it is predicted to become a huge concern in the coming years as the population ages, Males and females of 65 years and bygone account for 12% (36.8 million) of the population in the United States, a proportion that is predicted to double by 2030 [49].

Unfortunately, there are little substantial proof management recommendations for the diagnosis of urinary bladder cancer, particularly short- and long-term. Moreover, extrapolating based on research on younger individuals is inappropriate, younger particularly psychologically and socially and cancer increases the ranks of other while many aged persons suffering from age-related pathological conditions [50].

Intravesical therapy, particularly immunotherapy, would be less effective as individual's age, increasing the risk of complications. One analysis revealed a 10% absolute difference in liberation from disorder at 5 years even as Intravenous infusion BCG therapy for severe aged >70 years (27%) vs. those aged 70 years (37%), which was affirmed by a new study, which found a 22% lower incidence of illness for individuals aged >70 years (27%) [50].

2.7.3 Family History

A patient's risk of bladder cancer is enhanced if they have a family history of the disease, especially if they had first-degree relatives who were diagnosed with bladder cancer when they were 60 years old or younger. A family history of urothelial cell carcinoma has nearly doubled the risk among first-degree relatives of individuals with the disease[51].In certain exceptional cases, inheriting certain genetic diseases raises the risk. RB1 gene mutations cause retinoblastoma, PTEN

gene mutations cause Cowden disease, and MLH1, MSH2, MSH6, and PMS2 gene mutations Lynch syndrome, also referred as hereditary non-polyposis colorectal cancer, is a hereditary non-polyposis colorectal cancer.

Among the genetic different variables in bladder cancer carcinogenesis, variation in genes coding for xenobiotic bio transforming enzymes such as N-acetyltransferase 2 (NAT2) and glutathione S-transferees M1 (GSTM1) has received enormous attention [52]. Indeed, for this cancer, the strongest argument for an involvement of low-penetrance genetic variations in both raising cancer risk and gene-environment interactions occurs, Indeed for this cancer, the strongest argument for an involvement of low-penetrance genetic variations in both raising cancer risk and gene-environment interactions occurs and increased risk of bladder cancer has been correlated to a family history of various cancers like, lymphoblastic leukemia, cervical cancer, pancreas cancer, kidney cancer, thyroid cancer, and nervous system cancer [53]. While a very few of epidemiologic publications have investigated into the familial risk of bladder cancer, the results have been contradictory. While there have been a limited number of epidemiologic research exploring the familial risk of bladder cancer, early age of onset in propends and aggregation of cases within families are consistent with a genetic etiology [54]. According to a research of family assemblage of urothelial cell carcinoma (UCC), a prominent type of bladder cancer, first-degree relatives of UCC propends had a significantly increased incidence of developing UCC, indicating that UCC had a familial component, implying a tendency towards familial aggregation, family history is more contributing factor to developing bladder tumor [55].

2.7.4 Occupational Exposure

Bladder cancer is more common to develop when employees are exposed pesticides, pharmaceuticals, dyes, arsenic, aromatic polycyclic hydrocarbons, and chlorinated hydrocarbons. Paint products, rubber, leather, textiles, dye, metal, and petroleum product production units are among some of the companies that manufacture these compounds. Aromatic amines, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons are all substances that can be found in the workplace [56]. Bladder

cancer has been related to a variety of common industrial carcinogens. Bladder cancer is also a threat among workers in other industries who work with organic chemicals similarly Rubber, leather, and textile manufacturers are among those with increased risks [57].

2.7.5 Gender

Bladder cancer is the most frequent in males than in females. It is the seventh and seventeenth most prevalent malignancies in men and women worldwide respectively while males are three to four times more likely than females to acquire UCB although females have severe disease and have lower mortality rates. The dissimilarity among genders is believed to reflect genetic, anatomical, hormonal, social, and environmental factors, as well as a variation in carcinogen exposure (tobacco and chemicals)[58]. While males are affected by UCB at a higher rate than females, males have such a better prognosis. Males are approximately 3 to 4 times more likely than females to develop the disease. Men have a lower ratio of UCB-specific mortality to incidence than women. African-American females suffered the very worst, according to the researchers, data on patient age and tumor features verified prior studies that females with advanced phase. Differences in these prognostic variables, on the other side, only explained more than 30% of the difference in death rate among males and females, This suggests that the more prominent contributor with higher death rate in women delay in diagnosis, as indicated by the advanced stage of the disease [59].

2.7.6 Diet and Medical History

A 2018 meta-analysis found that individuals who eat more processed red meat having higher incidence of developing bladder tumor. Bladder tumor may not be increased by unprocessed red meat, contemporary diets have also associated with higher risk of developing bladder tumor recurrence [60]. Diets rich in fruits and vegetables and low fat diets minimize the risk of bladder cancer, whereas dietary retinol, beta-carotene, and meat all raise the risk, have really no impact on the

cancer, also investigated the potential and dietary fat has a significant correlation, example of this correlation , if persons who eat high-fat diets simultaneously eat low-fat diets a protective impact of fruits and vegetables may lead to a misleading causal effect associated to fat [61].

The diabetic drug pioglitazone is associated with bladder cancer greater risk. According to US and FDA, when using bigger doses the risk appears to increase while Aristolochic acid (found mostly in plants from the Aristolochia family) via dietary supplements have associated to an greater chance of urothelial malignancies, including bladder cancer, Cyclophosphamide, an anti-cancer medication, raises the risk of bladder cancer. Bladder cancer is the most frequently in people who had pelvic radiation for a previous cancer and squamous cell bladder cancer can be aggravated by recurring urinary infections or inflammations (cystitis), such as those triggered by long-term catheter use. In various parts of the world, squamous cell cancer has been implicated to schistosomiasis, a parasitic infection leads to chronic bladder inflammation [62, 63].

2.7.7 Previous Cancer Treatment and Genetics

Urothelial carcinomas can develop in the bladder, the kidney, the ureters, and the urethra, among several other places. If cancer in the lining of urinary tract it's more likely to develop elsewhere in the urinary tract. If the tumor at first stage is totally eliminated, persons who had bladder cancer should undergo annual checkups to ensure that no new malignancies have developed [64]. In primary non-muscle-invasive malignancy of bladder, renal ailments are major risk factor for tumor recurrence, progression, and overall survival. According to a report provided by the European Association of Urology in 2013, 30–80 % with NMIBC would experience recurrence, with a small percentage progressing to muscle-invasive malignancy within 5 years, depending on the financial characteristics [65].

Bladder cancer has been linked to urinary tract infections, renal and gall abnormalities, and other bladder irritation disorders. Schistosomiasis is a parasite illness that can enter the bladder and is linked to greater chance of squamous cell bladder

cancer. Schistosomiasis is extremely infrequent in US of America. The parasite is most prevalent in Africa and Middle East [64]. Other regions of the urothelial, such as the kidney lining, ureter, and urethra, can develop cancer any of these cancer types can increase the chances of another tumor developing in this layer of cells, Because secondary tumors in the urothelial are so common in people having bladder cancer [66].

Bladder cancer, particularly invasive squamous cell carcinomas, is linked to chronic urinary tract infection. Patients with spinal-cord injuries seem to be more prone to developing this type of cancer, as prolonged cystitis is a condition. This could be susceptible to bacterial flora forming nitrites and nitrosamines, as well as the inflammatory process, which causes increased cell proliferation [67].

N-acetyltransferase 2 (NAT2) slow acetylator and glutathione S-transferase 1 (GS TM1) - null genotypes were reproduced and considered to have significant correlations with bladder cancer risk [68]. The NAT2 slow acetylator phenotype confers an increased risk for malignancies where N-acetylation is a prominent detoxification mechanism, such as aromatic amine-related bladder cancer, because they have a lower capacity to detoxify aromatic amines through N-acetylation. The NAT2 slow acetylator genotype is correlated with the severity of bladder cancer [69].

Phase II enzymes known as glutathione S-transferase are essential in the detoxification of carcinogens detected in the environment. GSTM1 is involved in the detoxification of a number of carcinogens, including PAHs including benzopyrene. The GSTM1-null genotype was linked to a 50% increased risk of bladder cancer. No other potential SNPs were linked to the risk of bladder cancer [70].

Genetic alterations may account for a considerable proportion of all bladder malignancies, despite their minimal relative risks, due to their widespread frequency in the population. Because the pathways and genes involved in carcinogen metabolism and host defenses are well known, several investigations have investigated at relationships between single nucleotide polymorphisms in these pathways and bladder cancer risk [71]. Enzymes metabolism have particular role in prognosis of bladder

cancer, different enzymes have carcinogenic role in bladder cancer while some have detoxification role.

2.7.8 Fruit and Vegetables

A significant consumption of fruits and vegetables has been related to a lower incidence of almost all malignancies, including bladder cancer, in randomized trials. However, the proof is contradictory because the bulk of nutrients and metabolites, including (pre)-carcinogens, are eliminated through the urinary system, a role for food in bladder carcinogenesis is conceivable [72].

Bladder cancer may be minimized by consuming a diet rich in fruits and vegetables. In a more recent meta-analysis, fruit consumption was associated to a decreased risk of bladder cancer, but not vegetable consumption. The benefits of fruits and vegetables, carotenoids, folate, and vitamins A, C, and E upon bladder cancer incidence were examined [73].

Plant products can contain carcinogenic pesticides; despite the reality that they are high in vitamins, minerals, and other bioactive compounds that may aid to reduce tumor. Pesticide residues or traces can sometimes be found after pesticides are used to increase crop yield and influence of vegetables and fruits on the incidence of tumor bladder may muddled by pesticides in fruits and vegetables having a detrimental effect. Animal studies, on the other hand, are the only source of evidence for pesticide carcinogenic potential [74].

2.7.9 Familial Bladder Cancer

In comparison to the family incidence of many other tumor sites, familial bladder cancer is a relatively unusual occurrence. However, a number of sight case reports indicated family bladder cancer clustering [75]. Several of them have an exceptionally young onset age, suggesting a hereditary component and the incidence of bladder tumor is almost 2 times more than in first-degree relative with bladder cancer, according to epidemiological research from the Netherlands and Spain.

There is probably a lot of variation in risk, with a rare but highly penetrant gene being the culprit in some high-risk families [76].

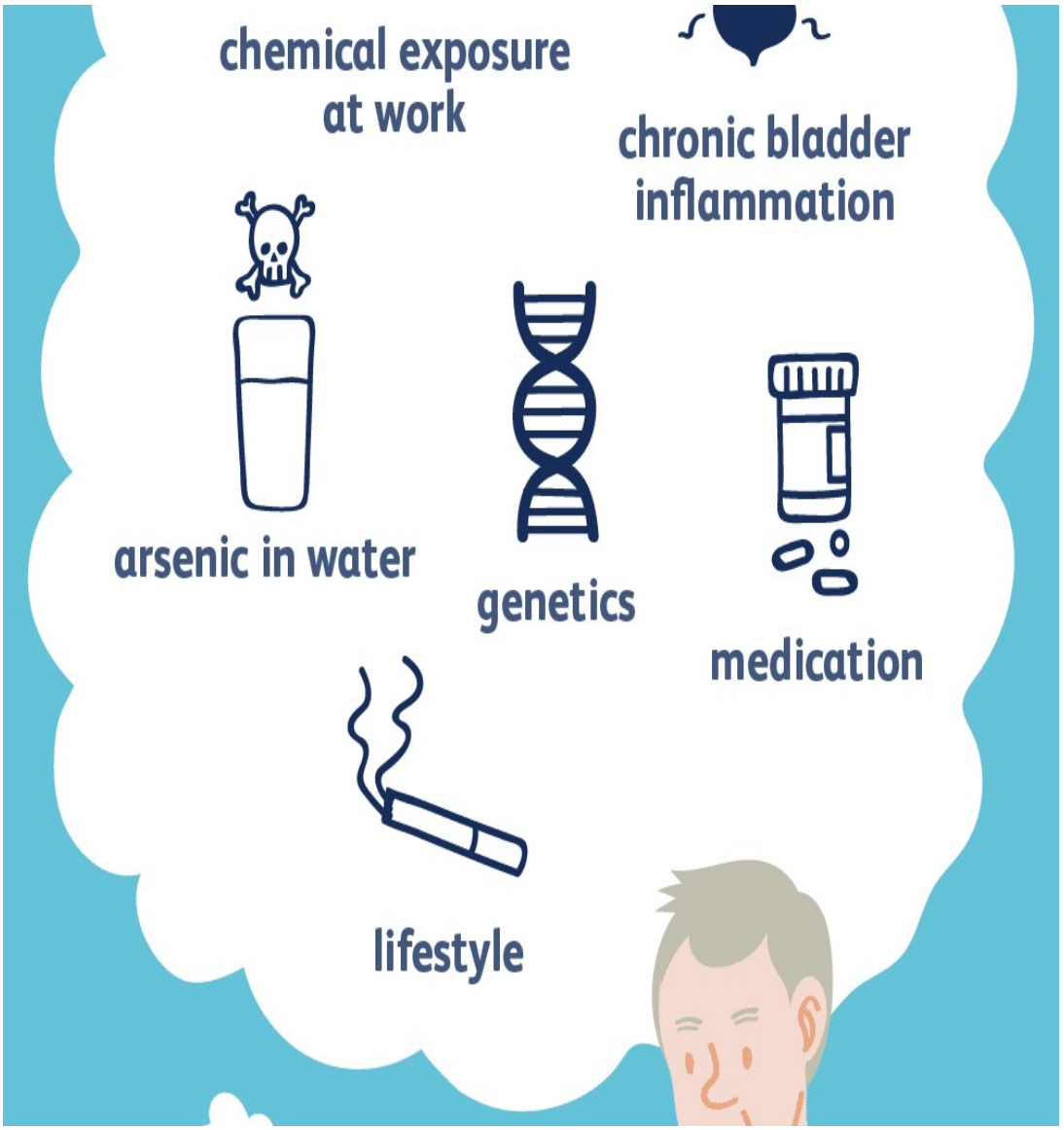


FIGURE 2.2: Risk Factors Associated with Bladder Cancer [76]

2.8 Treatments for Bladder Cancer

The variety, phase, and grading of the cancer, cancer biology (frequency, aggressiveness, and propagating capability), clinical manifestations (overall health and quality-of-life constraints), and patient - centered communication all decide the success of bladder cancer care (selection and type). Therapy is still most frequent

therapy for bladder tumors with acute, recurring, or distant metastases, and findings suggest that surgical measures such as surgical quality enhance bladder cancer prognosis [77].

2.9 Bladder Cancer Surgery

A TURBT (transurethral resection of bladder tumor) is a treatment that is done to see if there are any further tumors in the bladder and if present to eliminate them, if the cancer has progressed into (invaded) the muscular propria. This treatment perhaps more frequent therapy of premature or peripheral (non-muscle metastatic) genital malignancies. Whereas most individuals have marginal cancer when they are initially identified, a second, more comprehensive TURBT may be necessary to confirm that all cancer cells and underlying tissue have been removed all the way down to the bladder wall's muscularis propria [78].

2.9.1 Cystectomy

When bladder cancer progresses, may be required to eliminate the whole bladder or a section of it. During a partial cystectomy, if the cancer has advanced to the muscular layer of the lamina propria but is not very large and confined to one area, it may be removed together with a portion of the bladder wall [79]. The evidence for radical orchiectomy was premised on cystoscopy and histology observations, which included cancer cell intrusion of the muscular layer or testicular stromal, increased, urothelial tumors involved with melanoma, melanoma neuropathy to intravenous chemotherapy or immunotherapy, or recurrent multifocal superficial disease refractory to repeat transurethral resection [80].

2.9.2 Intravesical Therapy for Bladder Cancer

During TURBT, parenteral therapy is typically recommended (transurethral resection of bladder tumor). The most common Intravesical immunotherapy for

treating early stage bladder cancer is Bacillus Calmette-Guerin, or BCG. Because pharmaceuticals injected this way preferentially damage the cells lining the inside of the urethra, Intravesical chemotherapy is administered for these early-stage malignancies [81]. Subcutaneous surgery management and continuous cytoablation or immunostimulation to eradicate any persistent malignancies. BCG exceeds a few anticancer drugs (thiotepa, epirubicin, and doxorubicin) in terms of preventing tumorgenesis (14–47 % reduced recurrence rate) and disease progression [82].

Regardless of the fact that antineoplastic injectable BCG immunotherapy seems to be more efficient than surgical procedure by itself, just 50% of elevated NMIBC people receiving BCG pharmacological treatments as suggested either by EAU and AUA recommendations. One of the main reasons for poor BCG therapy compliance was the occurrence of significant complications. Furthermore, only 16 % of patients on maintenance BCG were able to complete the whole 3-year immunotherapy program because to adverse reactions [83].

2.9.3 Chemotherapy for Bladder Cancer

Chemotherapy (chemo) with the use of pharmaceuticals to eradicate tumors. Before surgery, intravenous and systemic chemotherapy are used to try to shrink a tumor so that it is easier to remove and to reduce the possibility that the cancer will return; after surgery (or sometimes after radiation therapy. Adjuvant therapy is the term for this method of therapy that used, eliminate any cancerous tissue after other therapies have been completed and may minimize the risk of various diseases returning in the future. This may reduce the chances of the cancer recurring in the later [84].

Chemotherapy is still the only treatment option for metastatic cancer its goal is to relieve symptoms, enhance the living standards, and increase bladder TCC patients' survival. Treatment has made very minimum headway in bladder TCC patients, and the conventional MVAC regimen is still the most often used regimen, and has been for several years[85]. Chemotherapy medications can be administered alone or in combination, depending on the situation, the patient's overall

health, and in addition to when chemo is paired with radiation, the most frequently administered pharmaceuticals are Cisplatin plus fluorouracil (5-FU) and Mitomycin with 5-FU, while the most frequently used chemo-radiation pairings include gemcitabine and cisplatin, as well as dose-dense methotrexate [86].

2.10 Clinical Enzymology of Bladder Cancer

The indolequinone EO9 showed outstanding preclinical performance after intravenous drug delivery, however it failed to show clinical efficacy against a variety of tumours. The failure of EO9 in the clinic has been attributed to its quick pharmacokinetic clearance, which results in poor medication delivery to tumors.. if bladder malignancies have greater concentrations of NQO1and (NADPH), a critical enzymes in activating EO9 under aerobic conditions [87]. The first report of telomerase reactivation in BC was published in the 1990s. Telomerase is an RNA-protein complex that generates telomere DNA that repeats. Telomeres are special structures at the ends of chromosomes where DNA combines with particular proteins called a "cap" that preserves and ensure the integrity of the chromosome ends [88].

Squamous cell carcinoma of the bladder results from a chronic infection with Schistosoma haematobium. An increased risk of this cancer is correlated to the NAT2 slow acetylator and GSTM1 null genotypes [89]Human telomerase is made up of a core protein (reverse transcriptase, or hTERT) as well as a matrix RNA component (telomerase RNA, or hTR), which through other enzymes structure an effective effectiveness refers to the extent whose function is to extend centromeric DNA to innovative repetitions and thus reverse the progressive loss of sequences at the ends of chromosomes due to incomplete Homologous recombination [90]. Mitomycin C (MMC) is a chemotherapeutic antibiotic that is converted to cytotoxic metabolites by cellular reductases through a process called technology that allow medication activity.[91] Understanding the impact and toxicity of industrial/environmental toxins requires a thorough understanding of xenobiotic chemical metabolism in the liver and extra hepatic tissues. Oxidation processes facilitated

mostly by cytochrome P-450s can result in reactive electrophilic molecules during metabolic bio activation of xenobiotic.

Cytochrome P-450s can also act as a reducing pathway, resulting in radical intermediates that can combine with oxygen to form a large number of oxygen species (ROS), leading in oxidative stress. Other enzymes related to metabolism bio activation in extra hepatic tissues comprise prostaglandin H synthase (PHS) [92]. Endogenous chemicals such as steroids, particularly oestrogen and melatonin, have been identified to be oxidised by CYP1B1. Exogenous components such as carbonyl compounds and carcinogens were converted to active carcinogenic metabolites in a similar manner [93]. Furthermore, elevation of CYP1B1 affected tumour cells susceptibility to anti-cancer pharmaceuticals such paclitaxel and docetaxel, resulting in treatment resistance , CYP1B1's differential and tumor-specific expression offers a potential possibility for the creation of new anticancer therapeutic interventions [94]. According to their acetylation activity, NAT2 phenotypes are classed as either rapid, moderate, or slow acetylators. This NAT2 weak genotype is caused by reduced oxygenation efficiency and is homozygotes for mutant NAT2 genes. The NAT2 slow genotype has indeed been linked with an increased risk of bladder cancer, according to genetic research[95]. UGTs are both a type of phase-2 pharmacokinetic and pharmacodynamics catalyzing the glucuronidation of a wide range of endobiotics and foreign substances.

UDP-glucuronyltransferase 1A (UGT1A), UDP-glucuronyltransferase 2A (UGT2A), and UDP-glucuronyltransferase 2B (UGT2B) are also the three regionals of UGTs (UGT2B) [96]. Although malignant cells can develop proteins that catabolize glycosaminoglycan's, N-acetyl—D-glucosamine (NAG, EC 3.2.1.30), a lysosome protein and member of the glycosidase family, may be associated to malignancy. NAG has potential biomarker for breast and gastrointestinal neoplasms [96].

2.11 P53 Role in Bladder Cancer

The p53 gene and protein statuses both seem to be essential for the smooth cell cycle, apoptotic, and apoptotic response while researchers and others have reported

previously mutations in the p53 protein that lead in the loss of the tumor suppressor genes activity. Cancer of the bladder, breast, lung, ovary, and colorectal cancer have all been investigated to see if the p53 gene is functional [97].

It has the ability to modulate target gene expression in order to play many functions in cellular progression, such as transcriptional activation, senescence, apoptosis, or metabolic and cell cycle abnormalities. TP53 mutations are inherent in social malignancies, including malignancy. TP53 mutations were found in nearly half of the MIBC samples, and TP53 activity is downregulated in 76% of the specimen [98].

The TP53 mutation and three adjacent may act as genetic alterations in bladder cancer, promoting disease process and altering survival and treatment options. Recombinant TP53 also activates pathways involved in cancer start and development, contributing to a poor prognosis. Mutant TP53 has been found by many researchers to accelerate metastatic tumor cell proliferation and promote metastatic potential. Furthermore, in tumors, TP53 status has been demonstrated to alter chemotherapy and drug resistance[99]. Mutations in the p53 gene between exons 5 and 8, which comprise the encoded protein's DNA-binding domain, were examined for in malignancies.

Exon 6 primers also amplified the end of intron 5. Exons 5, 6, 7, and 8 of the p53 gene revealed fragments of 205, 173, 149, and 157 bp, correspondingly. PCR resonance frequencies was carried out in such a maximum size of 50 l containing 50–100 ng Plasmid DNA, 10 mMTris-HCl (pH 8.0), 50 mMKCl, 1.5 mM MgCl2, dNTPs (100 mM each), 0.5 U Taq DNA polymerase, and 15 pmol each primers in 10 mMTris-HCl (pH 8.0), 50 mMKCl[100]. The p53 glycoprotein in the nuclei of all body cells, in which it connects (binds) to Genome immediately.

Whenever a cell's DNA is destroyed by chemicals like toxic substances, radioactivity, UV rays or daylight, this enzyme determines if the DNA will be repaired or the injured cell will self-destruct (undergo apoptosis). If the Gene mutation is recoverable, p53 triggers other genes to replace it. This antibody hinders the body from replicating and signals apoptosis if the DNA can't be repaired [101]. In certain

instances of malignancy, endogenous TP53 gene mutations have been identified. Tumor is a disorder in which mutated lymphocytes in the bladder develop rapidly, resulting in a tumor. Blood in the urine, pain when urinating, incontinence, the need to urinate but not being able to, and lower spine pain are all symptoms of malignancy [10]. The majority of these mutations in p53 affect only one amino acid. In cells with mutant or damaged DNA, this altered p53 protein is unable to control cellular activity, as well as cause apoptosis. Like a consequence, cells may develop Genetic mutations. carcinoma if these cells continue to divide excessively [10].

2.12 Prognosis of Bladder Cancer

The frequent occurrence of malignancy, and the necessity of continuous maintenance, places a burden on research to investigate early detection and prediction measures that would help them effectively manage the illness. The development of genetic markers enabling prognosis would minimize disease-related death and disability, as well as allow for more efficient use of medical resources through the implementation of individualized diagnostic and therapeutic strategies. Urine enters into intimate contact with urinary endothelial cells, which can cause BC. Urine, unlike other bodily fluids like blood, is collected transracial and in vast quantities. Urine proteomic has emerged as a viable and promising technique for identifying indicators for BC. Proteomic is an extremely new scientific domain for the research of physiological activities that require metabolites [13].

Gluconeogenesis and beta-oxidation were linked to these 12 intermediates ,used as ultra-HPLC-MS/MS (UHPLC-MS/MS) and chemical analysis spectrometry (GC-MS) to examine urine samples and developed a metabolite panel that could distinguish malignancy from non - malignant individuals. In addition, the diagnostic significance of urinary proteomic as both a marker for malignancy, that may be influenced by hemoglobin metabolites in the urine. When developing research to obtain useful diagnostic data, perturbations in these components must be controlled. However, while looking for metabolic indicators in recent investigations,

these differences have not been taken into account. Due to the absence of reliable diagnostic biomarkers, survival rate for elevated patients range from 30 to 70%, with risks of recurrence to lower - body malignancy (MIBC) as high as 10–30%. 2,3. [104].

Such issue necessitates a careful strategic approach involving periodic facilitator encouraged tests and a variety of therapeutic options. Although the FDA's acceptance of many urinary and plasma diagnostic tests for tumour screening, facilitator encouraged and urine histology remain the gold conventional diagnostic techniques for urinary cancer screening and monitoring. The diagnosis of tumours (BC) is determined by its phase and grade; the life expectancy for third level is higher. Since no biomarkers for malignancy have been identified, a condition is diagnosed entirely on the basis of microstructural analysis of tissue samples lesions and quantitative rating of cancer type and phase, on the other hand, indicates inter observer variability [105].

The distinction between reactionary invasive lobular adenomas in symptomatic urocystitis and Trans can be complicated, particularly within a week of treatment regimen with inducible microbes for purposeful initiation of a pro inflammatory, that is a principal effective therapy for Trans people aside from beginning surgical intervention.

The distinction between reactionary invasive lobular adenomas in symptomatic urocystitis and Trans can be complicated, particularly within a week of treatment regimen with inducible microbes for purposeful initiation of a pro inflammatory that is a principal effective therapy for Tran's people aside from beginning surgical intervention [106].

Chapter 3

Material and Methods

This study was done in Foundation Hospital Rawalpindi ,Shifa International Hospital Islamabad, Pakistan and Pakistan Institute of Medical Sciences (PIMS). Data was collected from patients of bladder tumor which were admitted in these hospitals and, also from the previous records of patients which were saved in Hospital Management System (HMS) of hospitals that are mentioned above. Questionnaire was filled out by asking different questions from patients and their MRI reports.

3.1 Approval of Study from Ethical Committee

For this clinical research study, approval from ethical committee of Capital University of Science and Technology (CUST) Islamabad was obtained. Approval were acquired from PIMS, Foundation Hospital Rawalpindi and Shifa International Hospital Islamabad. Pakistan to guarantee the ethical rights of participants.

3.2 Criteria for Acceptance and Elimination

Acceptance criteria for the present study, histopathologically verified cases of bladder cancer were selected from june-july 2021. The exclusion criteria were patients having benign bladder hyperplasia (BPH) or other urinary tract diseases. The patients having tumor other than bladder organ were excluded from study. Patients

who were receiving treatment, radiotherapy or chemotherapy were included. Patients receiving treatment, such as radiotherapy or chemotherapy, were included in the study.

3.3 Sample Size

This study had a total of 58 patients, 50 of whom had bladder cancer and 8 of whom had prostate cancer. Those with prostate cancer were excluded only patients with bladder cancer were considered [86].

3.4 Research Instrunments

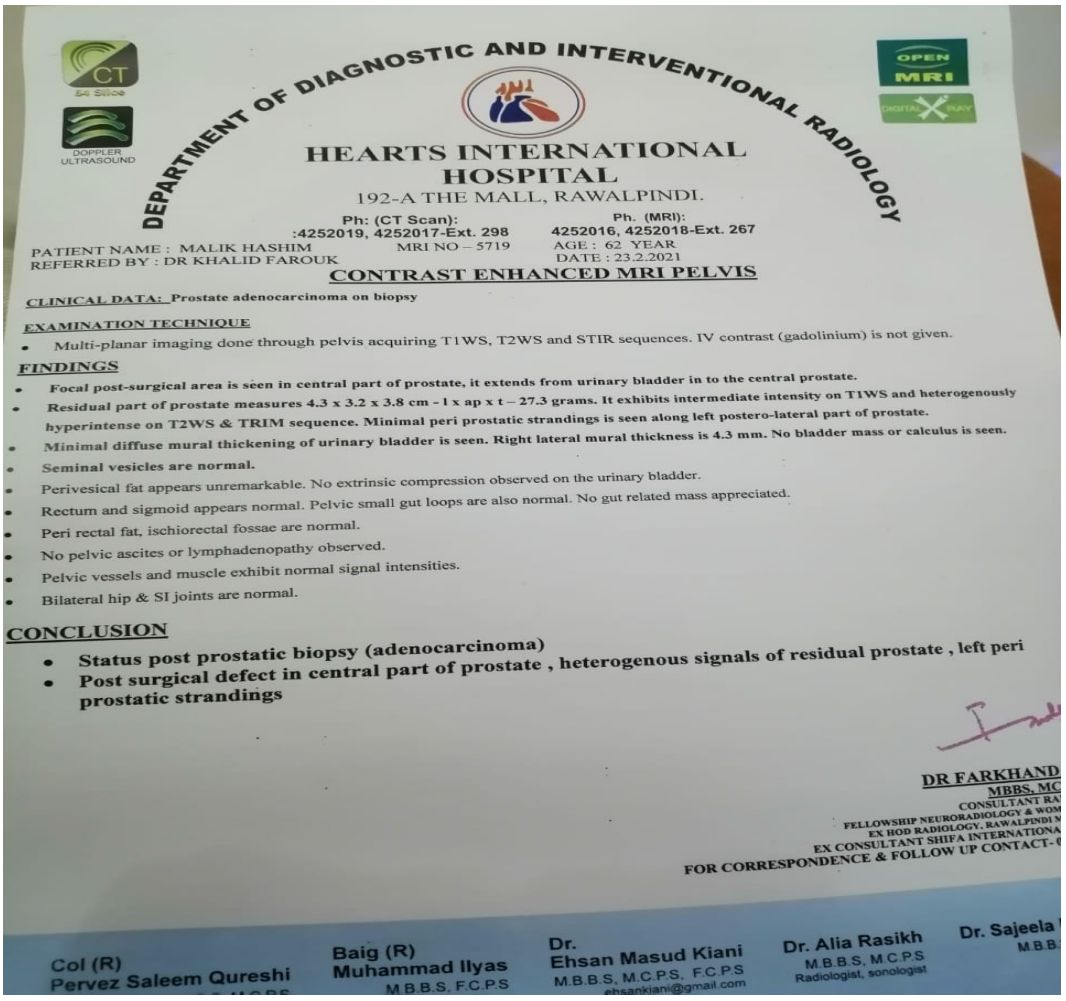
The standard questionnaire was constructed to collect data about the patient's medical history. All of the data was obtained from hospitalised patients and patient files. The patient's name, age, weight, height, area, gender, family history, dietary history, medical history, histopathological type of cancer, MRI results, ultrasound reports of patients, and metastatic organ were all documented on the questionnaire. Patients with lower urinary tract cancer and those who have experienced abdominal surgery in the past are considered in their medical history. Patient's with any medical condition who have been on medication were also identified. Patients' names and histories were withdrawn from the diagnosis list in June 2021, and files were then retrieved from the file room. Appropriate data was collected from files in order to accomplish this questionnaire. Shifa International Hospital had maintained patient's history in the record room and updated on the system. Patient's data were acquired from record room. Data was also gathered from the Foundation Hospital Rawalpindi. Data was collected from patients who were admitted starting in june 2021, and information for the questionnaire was filled out by patients and doctors from the Foundation Hospital's urology department. The ubiquitous risk factor for developing bladder cancer was identified using a logistic model and odds ratio, and the association with bladder cancer was validated using a chi square test.

FIGURE 3.1: Data Collection from Foundation Hospital Rawalpindi

Figure 3.2: Data Collection from PIMS

Figure 3.3 Data Collection from Shifa International Hospital Islamabad

FIGURE 3.3: Data Collection from Shifa International Hospital Islamabad



DEPARTMENT OF DIAGNOSTIC AND INTERVENTIONAL RADIOLOGY

HEARTS INTERNATIONAL HOSPITAL
192-A THE MALL, RAWALPINDI.

Ph: (CT Scan): :4252019, 4252017-Ext. 298
Ph. (MRI): 4252016, 4252018-Ext. 267

PATIENT NAME : MALIK HASHIM
REFERRED BY : DR KHALID FAROUK
MRI NO – 5719
AGE : 62 YEAR
DATE : 23.2.2021

CONTRAST ENHANCED MRI PELVIS

CLINICAL DATA: Prostate adenocarcinoma on biopsy

EXAMINATION TECHNIQUE

- Multi-planar imaging done through pelvis acquiring T1WS, T2WS and STIR sequences. IV contrast (gadolinium) is not given.

FINDINGS

- Focal post-surgical area is seen in central part of prostate, it extends from urinary bladder in to the central prostate.
- Residual part of prostate measures 4.3 x 3.2 x 3.8 cm - l x ap x t – 27.3 grams. It exhibits intermediate intensity on T1WS and heterogenously hyperintense on T2WS & TRIM sequence. Minimal peri prostatic strandings is seen along left postero-lateral part of prostate.
- Minimal diffuse mural thickening of urinary bladder is seen. Right lateral mural thickness is 4.3 mm. No bladder mass or calculus is seen.
- Seminal vesicles are normal.
- Perivesical fat appears unremarkable. No extrinsic compression observed on the urinary bladder.
- Rectum and sigmoid appears normal. Pelvic small gut loops are also normal. No gut related mass appreciated.
- Peri rectal fat, ischiorectal fossae are normal.
- No pelvic ascites or lymphadenopathy observed.
- Pelvic vessels and muscle exhibit normal signal intensities.
- Bilateral hip & SI joints are normal.

CONCLUSION

- **Status post prostatic biopsy (adenocarcinoma)**
- **Post surgical defect in central part of prostate , heterogenous signals of residual prostate , left peri prostatic strandings**

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Dr. Alia Rasikh M.B.B.S, M.C.P.S Radiologist, sonologist
Dr. Sajeela M.B.B.

FIGURE 3.4: MRI Reports of Bladder Cancer Patients

3.5 Statistical Analysis

The sample size for this study was 50. The data were collected from the patients of different hospitals of twin cities of Pakistan. A well-designed questionnaire was used as data collection tool. The questionnaire was based on open-end as well as closed-ended questions with 2 responses “Yes” and No”. The data recorded were converted on MS Excel 2010 for statistical analysis. The collected data regarding different epidemiological factors was analyzed by statistical analysis (SPSS software version14.0). All the data was summarized and arranged according to SPSS software. The SPSS version 26 was the data analysis tool. To fulfill the objectives and hypotheses of the study, the demographic profile of respondents was required. Descriptive statistics was obtained including minimum, maximum,

mean and standard deviation for average age, height and weight of respondents. The odds ratios were obtained to estimate the risk of bladder cancer with respect to the sex, smokers and the respondents who had been gone through any abdominal surgery. The chi-square statistics was obtained to find the association between different epidemiological (area and smoking) with bladder cancer.

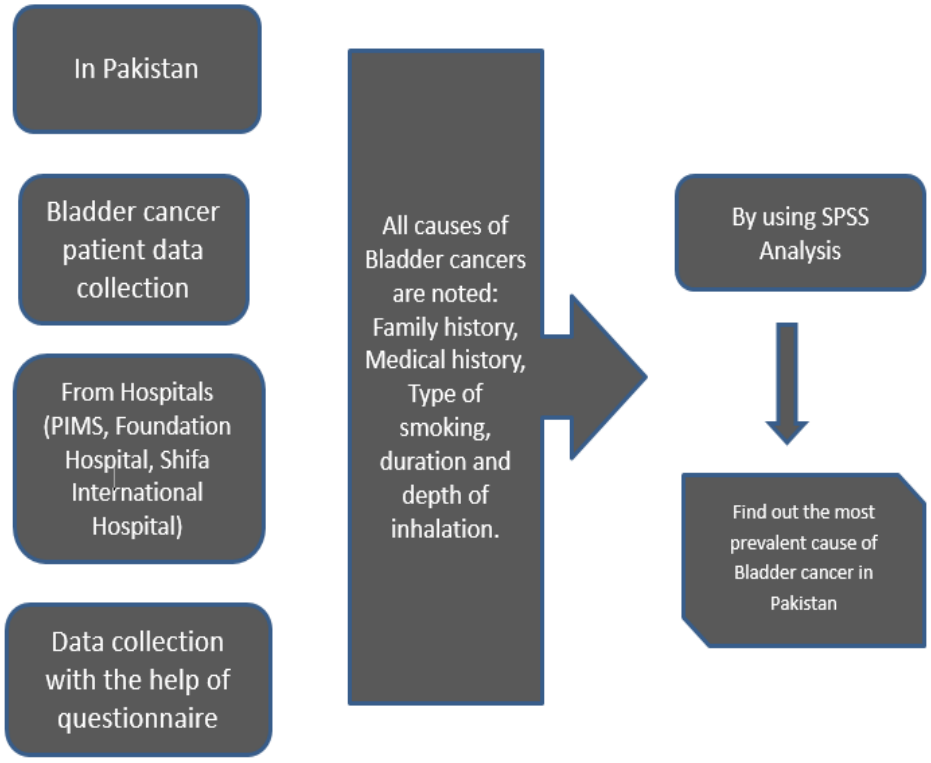

FIGURE 3.5: Flow chart, Methodology adopted to perform to research

Chapter 4

Results and Discussions

The statistical analysis that was used to construct this chapter. The most significant risk factors for bladder cancer were investigated using both descriptive and analytical methods in this chapter. To predict the various risk factors associated with bladder cancer, testing methods such as logistic ratio and chi square would be used.

4.1 Age, Weight and Gender

A total of 50 people took part in the survey and answered it. Males comprised 86% of the group, while females comprised 14%. Males' average age was 59.42 years old, with a standard deviation of 13.52, their average weight was 66.07kg, with a standard variation of 8.78. Females' average age was 56.14 years old with a standard deviation of 14.50, whose average weight was 57.86kg with a standard deviation of 7.

In case of females minimum age is 38 years while the maximum age is 80 years. By using descriptive statistics is noted in both males and females bladder cancer is disease of older age. In this age, height, weight and sex is taken as continuous variable and found bladder cancer is more prevalent among the patients with $\geq$50 age. Among 65-75 bladder cancer more respectively to those which have age of 40-45. Bladder cancer is most frequent in males compared to females.

TABLE 4.1: Descriptive Statistics

Sex		**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Male	Age	43	22	82	59.42	13.52
	Weight	43	38	80	66.07	8.78
Female	Age	7	38	80	56.14	14.5
	Weight	7	50	69	57.86	7.82

4.2 Residential Area and Area of Employment

Table 2 was obtained for the demographic profile of respondents. **Table 2** shows there were 43 (86%) out of total respondents were males and 7 (14%) out of total respondents were females. There were 14 (28%) out of total respondents whose age is between 71-80, 13 (26%) respondents were between 51-60 years old, 10 (20%) respondents were between 41-50 years old, 8 (16%) respondents were 61-70 years old, 3 (6%) respondents were between 31-40 and 1 (2%) of respondents were 21-30 and 81-90 years old. There were 22 out of total respondents lived in Rawalpindi which is the highest frequency of respondents, 6 from Chakwal and 5 from Kashmir. From the total respondents, there were 40 (80%) were employed and 10 (20%) were unemployed. There were 30 (60%) out of total respondents who were suffering from bladder cancer and 20(40%) respondents were not suffering from bladder cancer. It is noted from the given table of respondents residential area is not most leading cause of bladder cancer but the persons which are living near the areas which have higher exposure of arsenic have bladder cancer as compared to persons which are living in the areas where environment is somehow clean. Bladder cancer was diagnosed in people from various occupations in the survey, because the majority

of those admitted to hospitals are retired military advisers, the statistics revealed a higher incidence of bladder cancer among service members. Malignancy was not linked to the patient's occupation according to the statistics. Only those with activities that expose them to high levels of arsenic such as working in factories with high levels of arsenic are entitled [87].

TABLE 4.2: Demographic Profile of Respondents

		Frequency	**Percent**
Gender	Male	43	86
	Female	7	14
Age	21-30	1	2
	31-40	3	6
	41-50	10	20
	51-60	13	26
	61-70	8	16
	71-80	14	28
	81-90	1	2
Area	Attock	2	4
	Chakwal	6	12
	Faisalabad	1	2
	Jhang	1	2
	Kahuta	1	2
	Kallar syedan	1	2
	Kashmir	5	10
	Khaniwal	1	2
	Kharian	1	2
	Kohat	1	2

Continued Table: 4.2 Demographic Profile of Respondents

		Frequency	**Percent**
	Mandi bahawaldin	2	4
	Peshawar	2	4
	Rawalpindi	22	44
	Talagang	2	4
	Wah (cantt)	2	4
Employed	No	10	20
	Yes	40	80
Bladder Cancer	No	20	40
	Yes	30	60
	Total	**50**	**100**

4.3 Family History

In Table 4.3 frequency of Family history is calculated by descriptive statistics, percentage of respondents that are suffering from bladder cancer through 1 year are 16.0% while maximum percentage of respondents are those which are suffering from bladder cancer through 2 years is 36.0%. Percentage of respondents that are suffering from bladder cancer through 3 years is 34.0%. Loweset frequency is found of respondents that are suffering from bladder cancer through 4 years and 6 month and that is 2.0%. Respondants that are suffering from bladder cancer through many years that is represented as so long is 6.0%.

Time span of bladder cancer matter a lot, because with the passage of time others organs are metastasize, like kidney, prostate, pelvic girdle in case of males. In females same case those who are suffering from bladder cancer with very long period of time other organs are metastasize which include lamina propria, walls of bladder, and kidney. Bladder cancer is seen mostly in those respondents where bladder cancer runs in their family history. From given data 56.0% respondents are those in which have bladder cancers in their families. Maximum individuals are those which get bladder cancer through their grandparents that are 10(20%).

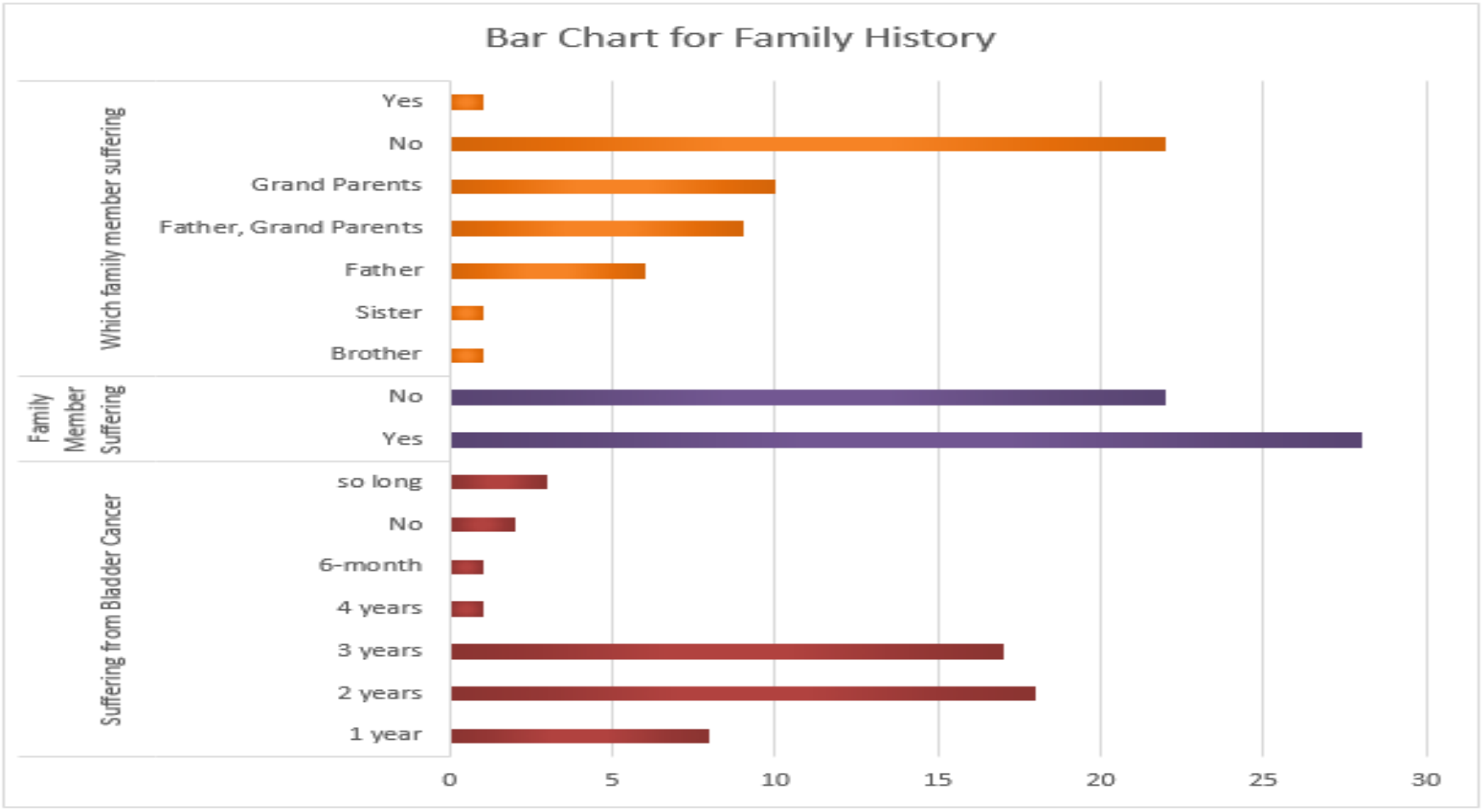

FIGURE 4.1: Bar Chart for Family History

TABLE 4.3: Family History

		Frequency	Percent
Respondents suffering	1 year	8	16
from Bladder Cancer	2 years	18	36
	3 years	17	34
	4 years	1	2
	6-month	1	2
	No	2	4
	so long	3	6
Family Member suffering	Yes	28	56
from Bladder Cancer	No	22	44
Family member of respondents	Brother	1	2
suffering from Bladder Cancer	Sister	1	2
	Father	6	12
	Father, Grand Parents	9	18
	Grand Parents	10	20
	No	22	44
	Yes	1	2
	Total	**50**	**100**

4.4 Dietary History

When the question was asked about their dietary habits it was found that mostly 90% respondents eat mix meal having meat and vegetables, while 2% take junk food, 4% meat and 4% only vegetables showing in table 4. The results show that food is not a factor which causes bladder cancer. 45(90.0) are those which eat everything like vegetables, meat and every type of food and 1(2.0%) are those which rely only on junk food and 2 (4.0) individuals are those which eat only vegetables but they develop bladder cancer.

TABLE 4.4: Dietary History

	Frequency	**Percent**
Junk Food	1	2
Meat	2	4
Mix	45	90
Vegetables	2	4
Total	50	100

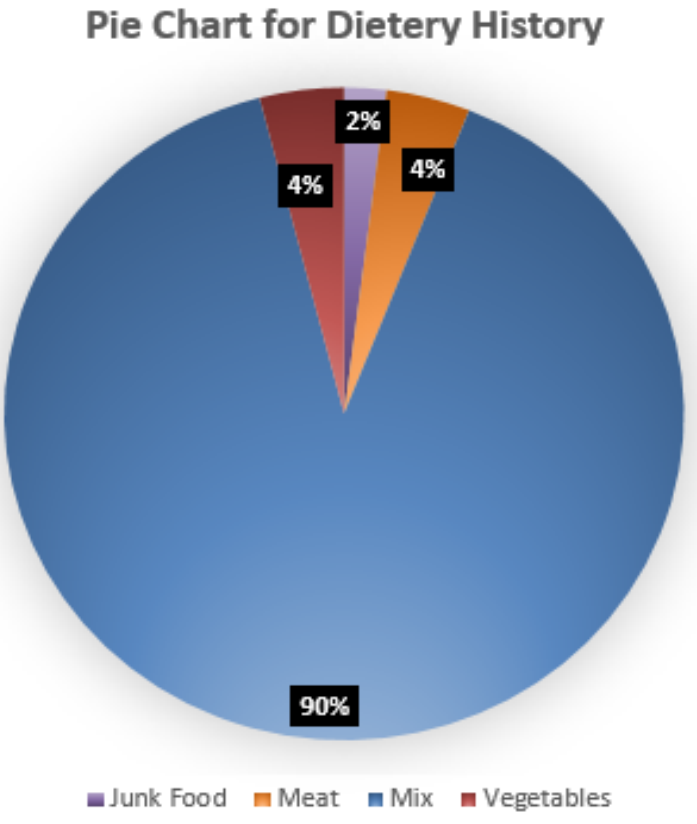

FIGURE 4.2: Pie Chart for Dietary History

4.5 Smoking History

In table 4.5, descriptive data are used to calculate the smoking history of bladder cancer respondents. 25 % in the table are smokers, including cigarette, naswar, and hukka addicts. Individuals who smoke 10-12 cigarettes per day comprise 8.0 %, while those using 2-3 and 3-4 cigarettes per day make up 2.0 %.14.0% individuals are those which are taking 4-5 to 6-8 cigarettes respectively. And 14.0% individuals are those which are taking 10 -12 cigarettes per day. From given table it is noted rate of bladder cancer is higher among persons who are taking many cigarettes per day. Similarly duration also matter a lot. In given tables chances of bladder cancer is equal among both respondents which are doing smoking for longer period of time and among those who start smoking 2-3-4 years respectively. Respondents 6-8 and 10-12 and duration of smoking both are 25%, both have equal chances of developing bladder cancer so it is illustrated that number and duration is not only risk factors of bladder cancer.

Table 4.5: Smoking History

	Frequency	**Percent**
More than 6 years	25	50.0
No	25	50.0
Cigarettes per day		
8-10 per day	4	8.0
2-3 per day	1	2.0
3-4 per day	1	2.0
4-5 per day	2	4.0
6-8 per day	7	14.0
10-12 per day	7	14.0
No	28	56
Total	50	100.0

4.6 Medical History

Descriptive statistics were used to calculate the medical histories of respondents in table 6. 80% of those polled encountered medical issues prior to contracting bladder cancer. Respondents suffer from a variety of medical conditions, including diabetes, high blood pressure, allergies, hypertension, and heart disease. These conditions do not play a significant role in the development of bladder cancer. Before developing bladder cancer, 20% of people have no medical problems. Bladder cancer patients have different forms of cancers, according to descriptive statistics. Adenocarcinoma concerns 29 (58.0%) of the people, carcinoma concerns 11 (22.0%), lymphomas concern 7 (14.0%), sarcoma concerns 2 (4.0%) of the people, and transitional cell carcinoma concerns just 1 (2.0%) of the people.

Table 4.6: Medical History

Medical Issue	**Frequency**	**Percent**
No	10	20
Yes	40	80
Addiction		
No	25	50
Yes	25	50
Type of Cancer		
Adenocarcinoma	29	58
Carcinoma	11	22
Lymphomas	7	14
Sarcoma	2	4
Transitional cell carcinoma	1	2
Total	50	100

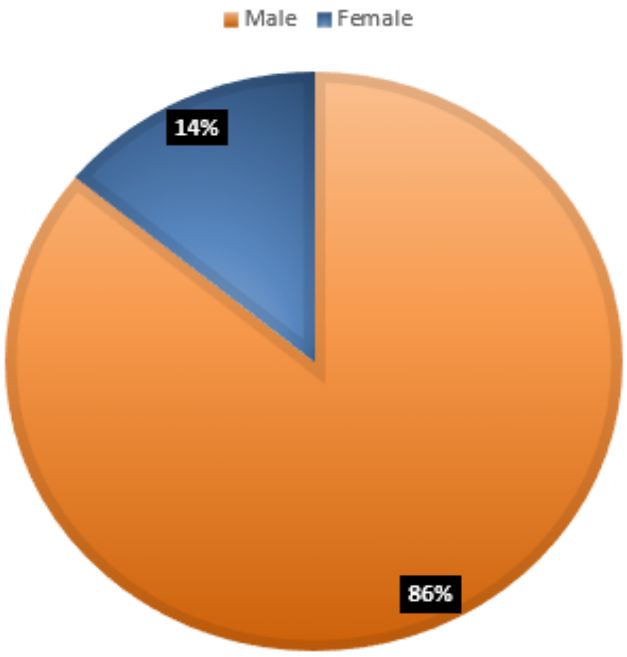

FIGURE 4.3: Pie Chart for Gender

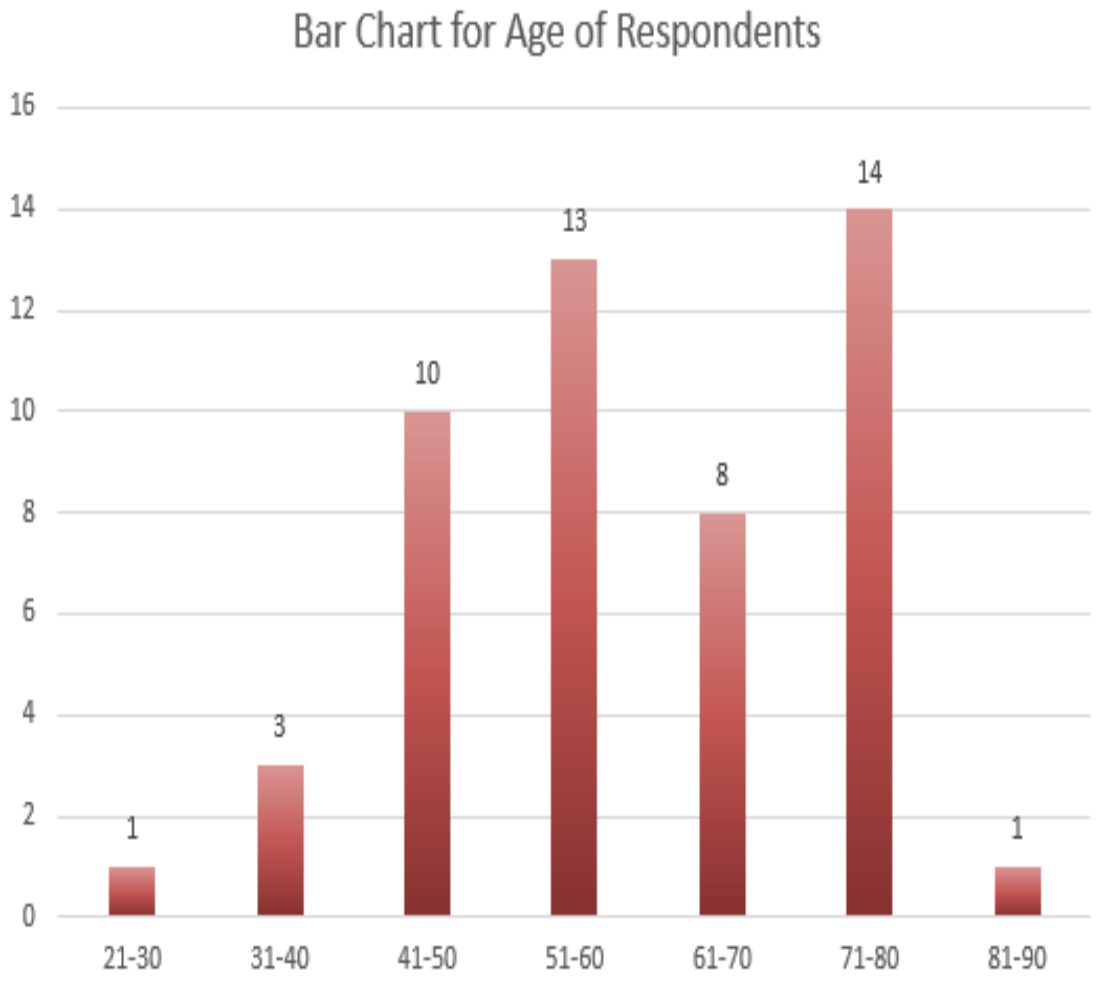

FIGURE 4.4: Bar Chart for Age of Respondent

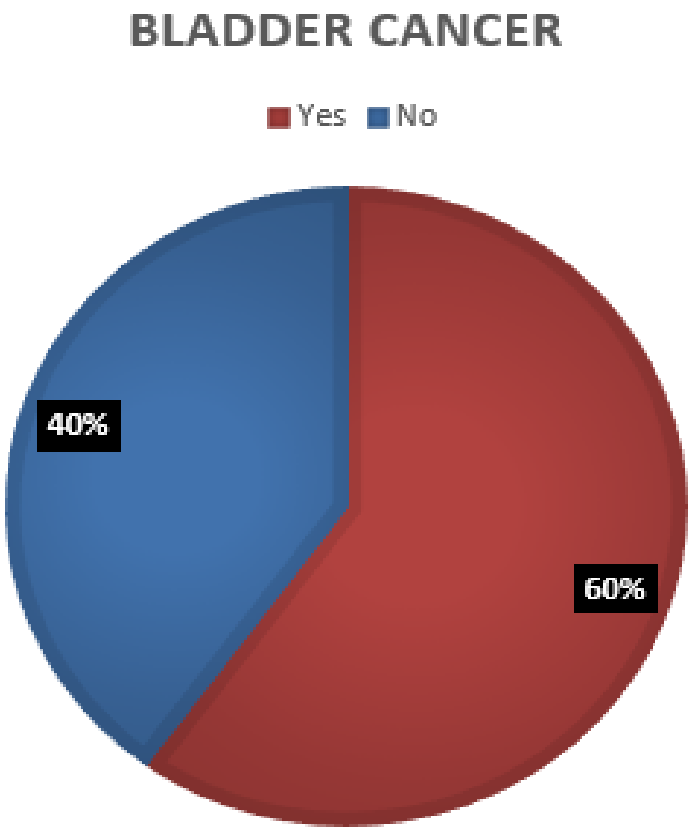

FIGURE 4.5: Pie Chart for Respondents who are Suffering from Bladder Cancer

4.7 Risk of Bladder Cancer (Different Epidemiological Factors)

To estimate the risk of bladder cancer with respect to sex, smoking and abdominal surgery the odds ratios were obtained. The logistic model for this study as follows:

$$Log(odds of Bladder Cancer) = \alpha + \beta_1[Sex = Male] + \beta_2[Sex = Female] + \beta_3[Smoking = Yes] + \beta_4[smoking = No] + \beta_5[AbdominalSurgery = Yes] + \beta_6[AbdominalSurgery = No]. \quad (4.1)$$

where β_2=0 as sex = female, β_4=0 as smoking = no and β_6=0 as abdominal surgery = No were taken as reference category

Table 4.7: Chi-Square Tests

		B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp (B)**	**95% C.I.for EXP(B)**	
								Lower	**Upper**
Step 1a	sex (1)	0.083	0.911	0.008	1	0.927	1.087	0.182	6.475
	Smoking (1)	-0.373	0.652	0.328	1	0.567	0.688	0.192	2.47
	Abdominal Surgery (1)	0.079	0.599	0.017	1	0.896	1.082	0.334	3.501
	Constant	-0.333	0.838	0.158	1	0.691	0.717		

a. Variable(s) entered on step 1: sex, Smoking, Abdominal Surgery.

Log (odds of Bladder Cancer) = -0.333+ 0.083[Sex=Male]-0.373[smoking=Yes] +0.79[Abdominal Surgery=Yes].

The Exponent (B) estimates represents the odds ratios for each of the variables provided in table 3. The odds of bladder cancer are 1.087 times higher in the males than the females follow up the 95% CI (.182, 6.475). The Exponent (B) estimates for Smoking (1) = .688 follows up the 95% CI (.192, 2.470) which indicates that the odds of bladder cancer are .688 time higher in the people who are smoker than that of who are non-smoker.

The Exponent (B) estimates for Abdominal Surgery (1) = 1.082 follows up the 95% CI (.334, 3.501), which implies that the odds of bladder cancer are 1.082 times higher in the patients who suffered from abdominal surgery than the patients who do not suffered from any abdominal surgery.

Different forms of smoking is considered as smoking which include naswar addiction, Hukka smokers have more chances of bladder cancer, while it is noted nonsmokers have very low chances of bladder cancer. From the given data is noted that any kind of smoking have more chances of bladder cancer.

4.8 Association of Different Epidemiological (Residential Area and Smoking)

The table 8, Indicates that there were 22 out of total respondents from Rawalpindi which is the highest frequency of respondents and 12 were suffering from bladder cancer whereas 10 were not. The second high frequency was found in Chakwal based on 6 out of total respondents from which, 4 were suffering from bladder cancer and 2 were not.

While in Talegang, Wah cantt and Attock have only 2 respondents from which one was suffering from bladder cancer and one was not. In case of Kashmir 5 respondents from which 2 were suffering bladder cancer while 3 were not.

TABLE 4.8: Area * Bladder Cancer Cross tabulation

		Bladder Cancer		
		Yes	**No**	**Total**
Area	Attock	1	1	2
	Chakwal	4	2	6
	Faisalabad	1	0	1
	Jhang	1	0	1
	Kahuta	1	0	1
	Kallar syedan	0	1	1
	Kashmir	3	2	5
	Khaniwal	1	0	1
	Kharian	0	1	1
	Kohat	1	0	1
	Mandi bahawaldin	2	0	2
	Peshawar	1	1	2
	Rawalpindi	12	10	22
	Talagang	1	1	2
	Wah(cantt)	1	1	2
Total		30	20	50

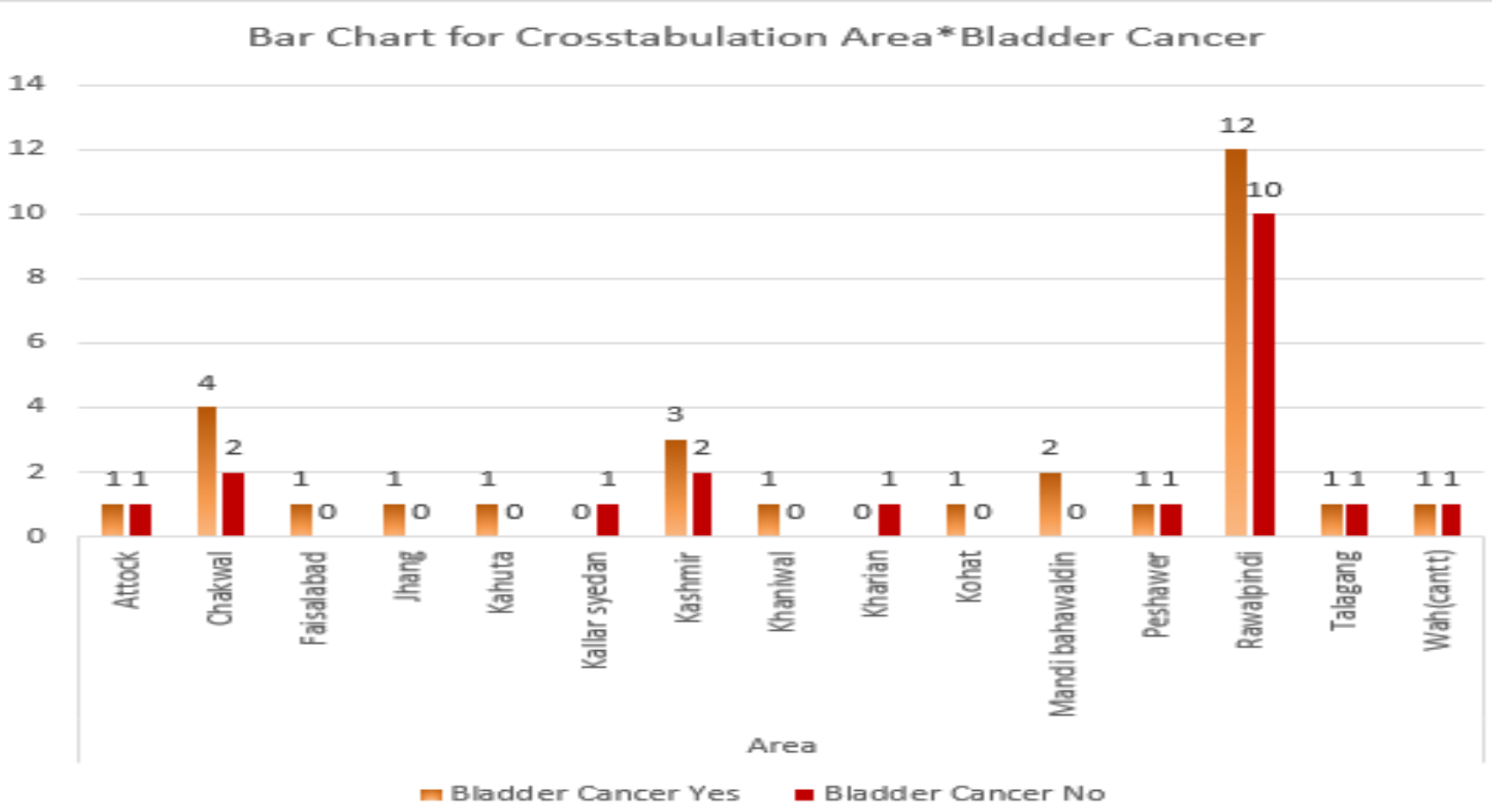

FIGURE 4.6: Bar Chart for Cross tabulation Area and Bladder Cancer

TABLE 4.9: Chi-Square Tests

	Value	**Df**	**Asymptotic (2-sided)**	**Significance**
Pearson Chi-Square	8.384a	14	0.868	
Number of Valid Cases	50			

a. 28 cells (93.3%) have expected count less than 5. The minimum expected count is .40.

The above table was obtained for the association between residential areas and bladder cancer. The results showed that there was statistically nonsignificant association between residential areas and bladder cancer ($p = .868 > .05$) which indicates that there is no relationship between residential area and bladder cancer.

TABLE 4.10: Smoking * Bladder Cancer Cross Tabulation

		Bladder Cancer		
		Yes	**No**	**Total**
Smoking	Yes	20	5	25
	No	10	15	25
Total		30	20	50

The results found in table 6 showed that out of total respondents 25 were smokers and 25 were non-smokers. 20 patients who are smokers and suffering from bladder cancer whereas 10 non-smokers were suffering from bladder cancer. Similarly, 5 smokers were not suffering from bladder cancer whereas 15 respondents were non-smokers and were not suffering from bladder cancer.

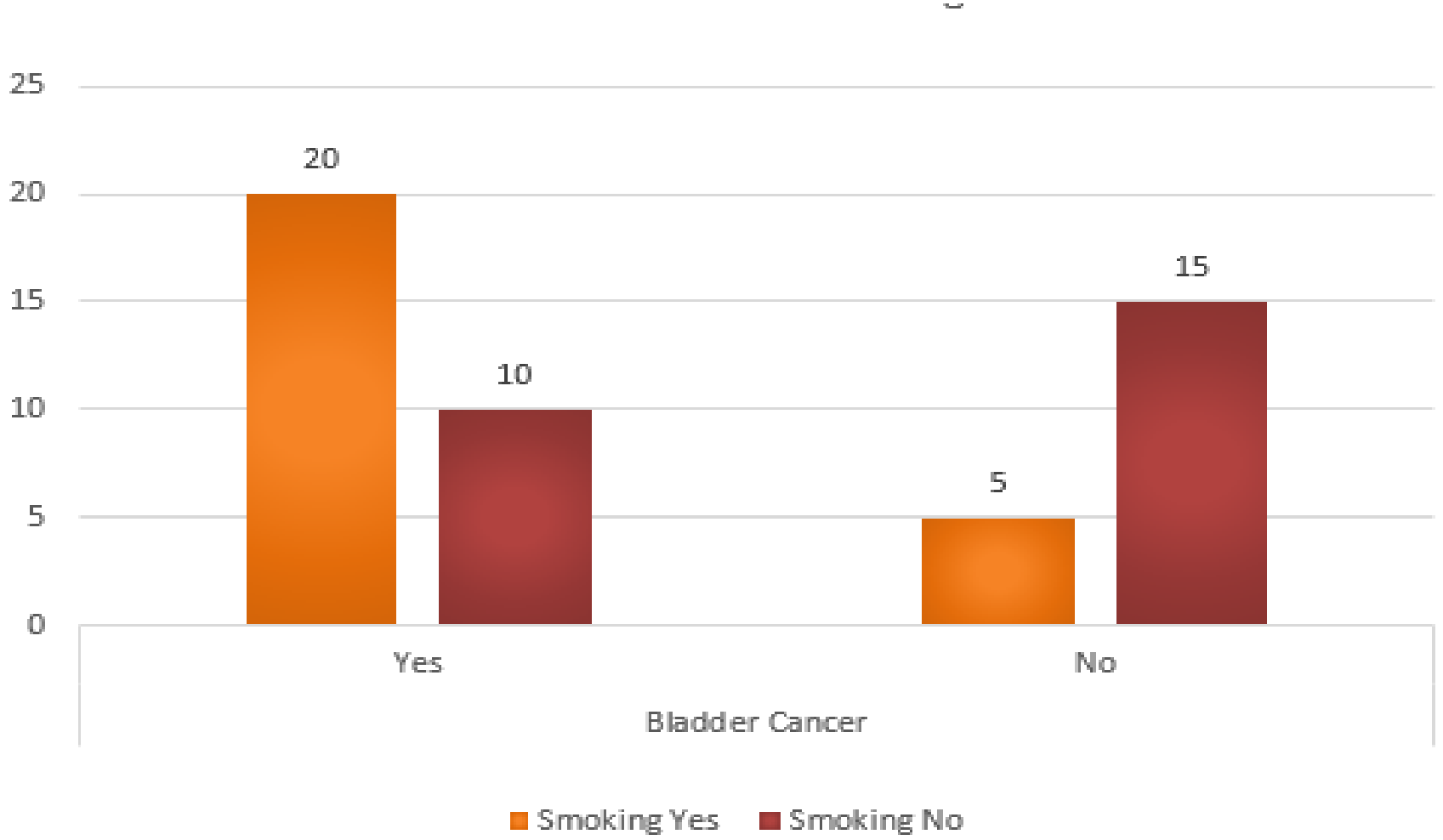

FIGURE 4.7: Bar Chart for Cross tabulation of Smoking and Bladder Cancer

TABLE 4.11: Chi-Square Tests

	Value	**df**	**Asymptotic Significance (2-sided)**
Pearson Chi-Square	8.3333	1	0.004
N of Valid Cases	50		

The above table was obtained for the association between smokers and bladder cancer. The results showed that there was statistically significant association between smokers and bladder cancer ($p = .004 < .05$) and indicates that the smoking is the major factor for the bladder cancer.

Chapter 5

Results and Discussion

Data were collected from different hospitals in Pakistan, including Foundation Hospital Rawalpindi, Pakistan Institute of Medical Sciences (PIMS), and Shifa International Hospital, to assess the most prevalent risk factor for bladder cancer in the Pakistani population.

Patients' histories were retrieved by asking patients and caregivers a series of questions. Age, gender, height, weight, area, family history, dietary history, medical history, histopathological type of cancer, and metastatic type of cancer are all provided on the questionnaire. As it is observed bladder cancer is higher among males as compared to females because, males smoke more than females and several carcinogens that are present in cigrratte smoke are responsible for the development of bladder cancer [88].

Bladder cancer seems to have a number of significant risk factors. It has been observed that the risk of bladder cancer is higher in patients who have had previous abdominal surgery before developing bladder cancer, and that people who have had more arsenic exposure have a higher risk of developing bladder cancer. Greater exposure to carcinogens in the environment, such as different patterns of employment or tobacco use, could explain the difference in risk between men and women. Our findings imply that if men and women were equally exposed to cigarette smoking, occupational risks, and urinary tract infections, the male-to-female risk ratio would be lowered, but that there would still be a significant risk differential between men and women [89].

With respect to age bladder cancer is now commonly regarded as primarily a disease of the elderly, with age commonly recognized as the greatest single risk factor. Because of the adequate backup between age and the prevalence of bladder cancer, it is predicted that as the older population, this cancer will become a major challenge [90].

When it comes to dietary habits, 2% of people eat junk food and 4% eat meat, whereas 90% of people eat a variety of foods including vegetables, meat, and junk food. Bladder cancer is more common in those who eat a wide range of foods and vegetables because different carcinogenf are present in their diet on a regular basis [91].

Tobacco use is estimated to be the major cause of urinary bladder cancer, with almost 50% of all patients having smoked at some stage of life. When compared to non-smokers, cigarette smokers had a four-fold increased risk of bladder cancer. With an odds ratio of 1.9 and a 95 percent confidence interval of 1.1-3.1, those who had smoked for less than 10 years had a two-fold higher risk than non-smokers. Those who have smoked for more than 40 years had similar 4.1 and 3.0-5.5 values. Residential area does not appear to be associated to urinary bladder cancer, indicating that it has no involvement in the cancer.

According to the chi square value of.004**, smoking also increases risk of bladder cancer than nonsmokers.The odds of bladder cancer are 1.087 times higher in males than in females, according to the 95 % confidence interval (.182, 6.475). The 95 % confidence interval (.192, 2.470) for the exponent (B) estimates for smoking (1) =.688 suggests that the risk of bladder cancer is.688 times higher in smokers than in non-smokers. Three variables, including sex, dietary habits, smoking, and residential location, were revealed to be significantly associated to bladder cancer through using Chi-square analysis. The value for abdominal surgery (1) = 1.082, with a 95 % confidence interval of (.334, 3.501), indicates that patients who have had abdominal surgery have a 1.082 times higher risk of bladder cancer than those who have not had abdominal surgery. This suggests that abdominal surgery may have a role in bladder cancer progression. To conclude, smoking over an incredibly long time, previous abdominal surgeries, and gender are all predicted

to be significant risk factors for bladder cancer. Individuals who are smoking for many years have greater risk of developing bladder cancer, Like the person who are taking 8-10 and 10-12 cigarettes per day are on greater risk of developing bladder cancer.

SPSS analysis was used to determine the most frequent occurrence of bladder cancer. To investigate the relationship between these two characteristics, family history and smoking, logistic regression and odds ratio were used. After obtaining an odd ratio and realizing that males are more likely than females to develop bladder cancer, explore the connection between smoking and family history of smokers and non-smokers. Applying logistic regression, it was revealed that smokers have a.373-unit higher risk of bladder cancer compared nonsmokers. Similarly, individuals who had abdominal surgery in the past had a 0.79-unit increased risk of bladder cancer than those who have not had abdominal surgery in the past. All of them are regression interpretations.

By using odd ratio we observed those respondents which are doing smoking for longer period of time and taking many cigarettes per day have higher risk of bladder cancer as compared to nonsmokers. When examining bladder cancer risk associated with smoking status stratified by number of cigarettes have greater risk as compared to those respondents which are taking minimum cigarettes consuming per day. In case of females they are not active smokers but they are passive smokers they have equal chances of bladder cancer such as in males active smokers. According to the Pakistan Cancer Society, bladder cancer is the 4th and 15th most prevalent cancer in men and women, respectively, and smoking is the primary factor of bladder cancer. Tobacco use is thought to be the leading cause of urinary bladder cancer, with around half of all patients having a smoking history. Cigarette smokers had a four-fold increased risk of bladder cancer as compared to non-smokers. Those who smoked for less than 10 years had a two-fold higher risk compared to non-smokers, with an odds ratio of 1.9 and a 95 percent confidence interval of 1.1-3.1. Those who had smoked for more than 40 years had similar values of 4.1 and 3.0-5.5, respectively. According to the findings, residential area is not related with urinary bladder cancer, indicating that there is no role of

residential area in bladder cancer. According to chi square .004** value indicating significant role of getting bladder cancer as compared to nonsmokers.

The odds of bladder cancer are 1.087 times higher in the males than the females follow up the 95% CI (.182, 6.475). The Exponent (B) estimates for Smoking (1) = .688 follows up the 95% CI (.192, 2.470) which indicates that the odds of bladder cancer are .688 time higher in the people who are smoker than that of who are non-smoker. The Chi-square found that three variables, including sex, dietary habits, smoking, residential area were substantially not related with bladder cancer.

The value for Abdominal Surgery (1) = 1.082 having 95% CI (.334, 3.501), which indicate that the odds of bladder cancer are 1.082 times higher in the patients who suffered from abdominal surgery than the patients who do not suffered from any abdominal surgery. It indicates that abdominal surgery has some role in causing bladder cancer.

Bibliography

1. Saginala, K., et al., Epidemiology of bladder cancer. *Medical Sciences*, 2020. 8(1): p. 15.

2. Jones, P.A. and S.B. Baylin, The epigenomics of cancer. Cell, 2007. 128(4): p. 683-692.

3. Smith, A.B. Recent developments in the management of bladder cancer: introduction. in Urologic Oncology: Seminars and Original Investigations. 2018. Elsevier.

4. Clark, P.E., et al., Bladder cancer. *Journal of the National Comprehensive Cancer Network*, 2013. 11(4): p. 446-475.

5. Shukur, H.Y., Gemcitabine Single Agent for Recurrent Post Bladder Preservation Therapy and in Metastatic Transitional Cell Carcinoma of Urinary Bladder in Elderly Patients with Renal Impairment. 2016.

6. Wong-You–Cheong, J.J., et al., Neoplasms of the urinary bladder: radiologic-pathologic correlation. Radiographics, 2006. 26(2): p. 553-580.

7. Ruddon, R.W., Cancer biology. 2007: Oxford University Press.

8. Kiemeney, L.A. and M. Schoenberg, Familial transitional cell carcinoma. *The Journal of urology*, 1996. 156(3): p. 867-872.

9. Burger, M., et al., Epidemiology and risk factors of urothelial bladder cancer. *European urology*, 2013. 63(2): p. 234-241.

10. Dmochowski, R. R. (2005). Bladder outlet obstruction: etiology and evaluation. *Reviews in Urology*, 7(Suppl 6), S3.

11. Torre, L.A., et al., Global cancer statistics, 2012. CA: *a cancer journal for clinicians*, 2015. 65(2): p. 87-108.

12. Torre, L.A., et al., Worldwide burden of and trends in mortality from gallbladder and other biliary tract cancers. *Clinical Gastroenterology and Hepatology*, 2018. 16(3): p. 427-437.

13. Jemal, A., et al., Cancer statistics, 2006. CA: *a cancer journal for clinicians*, 2006. 56(2): p. 106-130.

14. Abidine, Y., et al., Local mechanical properties of bladder cancer cells measured by AFM as a signature of metastatic potential. *The European Physical Journal Plus*, 2015. 130(10): p. 1-13.

15. Yamaguchi, H. and J. Condeelis, Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochimica et Biophysica Acta (BBA)-*Molecular Cell Research*, 2007. 1773(5): p. 642-652.

16. Bao, G. and S. Suresh, Cell and molecular mechanics of biological materials. *Nature materials*, 2003. 2(11): p. 715-725.

17. Yan, B., et al., Study of cholesterol repletion effect on nanomechanical properties of human umbilical vein endothelial cell via rapid broadband atomic force microscopy. *Journal of biomechanical engineering*, 2017. 139(3): p. 034501.

18. Faria, E.C., et al., Measurement of elastic properties of prostate cancer cells using AFM. *Analyst*, 2008. 133(11): p. 1498-1500.

19. Ricol, D., et al., Tumour suppressive properties of fibroblast growth factor receptor 2-IIIb in human bladder cancer. Oncogene, 1999. 18(51): p. 7234-7243.

20. LaRochelle, W.J., et al., Specific receptor detection by a functional keratinocyte growth factor-immunoglobulin chimera. *The Journal of cell biology*, 1995. 129(2): p. 357-366.

21. Park, S., et al., MacroH2A1 downregulation enhances the stem-like properties of bladder cancer cells by transactivation of Lin28B. Oncogene, 2016. 35(10): p. 1292-1301.

22. Bhurgri, Y., et al., Cancer incidence in Karachi, Pakistan: first results from Karachi cancer registry. *International journal of cancer*, 2000. 85(3): p. 325-329.

23. Hanif, M., et al., Institution-based cancer incidence in a local population in Pakistan: nine year data analysis. *Asian Pac J Cancer Prev*, 2009. 10(2): p. 227-230.

24. Bhurgri, Y., et al., Cancer profile of hyderabad, Pakistan 1998-2002. *Asian Pacific journal of cancer prevention*, 2005. 6(4): p. 474.

25. Bhurgri, Y., et al., Pakistan-country profile of cancer and cancer control 1995-2004. *Journal of the Pakistan Medical Association*, 2006. 56(3): p. 124.

26. Massari, F., et al., Metabolic phenotype of bladder cancer. *Cancer treatment reviews*, 2016. 45: p. 46-57.

27. Prasad, S.M., G.J. DeCastro, and G.D. Steinberg, Urothelial carcinoma of the bladder: definition, treatment and future efforts. *Nature Reviews Urology*, 2011. 8(11): p. 631-642.

28. Galsky, M.D., et al., A consensus definition of patients with metastatic urothelial carcinoma who are unfit for cisplatin-based chemotherapy. *The lancet oncology*, 2011. 12(3): p. 211-214.

29. Hurst, C.D., et al., Novel tumor subgroups of urothelial carcinoma of the bladder defined by integrated genomic analysis. *Clinical Cancer Research*, 2012. 18(21): p. 5865-5877.

30. Colombel, M., et al., Epidemiology, staging, grading, and risk stratification of bladder cancer. *European Urology Supplements*, 2008. 7(10): p. 618-626.

31. Volanis, D., et al., Environmental factors and genetic susceptibility promote urinary bladder cancer. *Toxicology letters*, 2010. 193(2): p. 131-137.

32. Kiriluk, K.J., et al. Bladder cancer risk from occupational and environmental exposures. in Urologic Oncology: *Seminars and Original Investigations*. 2012. Elsevier.

33. Jung, H., et al., Validation of the seventh edition of the American Joint Committee on Cancer TNM staging system for gastric cancer. Cancer, 2011. 117(11): p. 2371-2378.

34. Strosberg, J.R., et al., Prognostic validity of a novel American Joint Committee on Cancer Staging Classification for pancreatic neuroendocrine tumors. *Journal of Clinical Oncology*, 2011. 29(22): p. 3044-3049.

35. Satoh, H., et al., Intravesical ultrasonography for tumor staging in an orthotopically implanted rat model of bladder cancer. *The Journal of Urology*, 2007. 177(3): p. 1169-1173.

36. Levin, R. M., Monson, F. C., Haugaard, N., Buttyan, R., Hudson, A., Roelofs, M., & Wein, A. J. (1995). Genetic and cellular characteristics of bladder outlet obstruction. *Urologic Clinics of North America*, 22(2), 263-283.

37. Kaufman, D.S., W.U. Shipley, and A.S. Feldman, Bladder cancer. *The Lancet*, 2009. 374(9685): p. 239-249.

38. Sharma, S., P. Ksheersagar, and P. Sharma, Diagnosis and treatment of bladder cancer. *American Family Physician*, 2009. 80(7): p. 717-723.

39. Carmack, A.J. and M.S. Soloway, The diagnosis and staging of bladder cancer: from RBCs to TURs. *Urology*, 2006. 67(3): p. 3-8.

40. Henningsohn, L., et al., Distressful symptoms after radical radiotherapy for urinary bladder cancer. *Radiotherapy and Oncology*, 2002. 62(2): p. 215-225.

41. Mungan, N.A., et al., Gender differences in stage distribution of bladder cancer. Urology, 2000. 55(3): p. 368-371.

42. Salehi, A., et al., Epidemiologic status of bladder cancer in Shiraz, southern Iran. *Asian Pac J Cancer Prev*, 2011. 12(5): p. 1323-7.

43. Idrees, R., et al., Cancer prevalence in Pakistan: meta-analysis of various published studies to determine variation in cancer figures resulting from marked population heterogeneity in different parts of the country. *World journal of surgical oncology*, 2018. 16(1): p. 1-11.

44. Janković, S. and V. Radosavljević, Risk factors for bladder cancer. *Tumori Journal*, 2007. 93(1): p. 4-12.

45. Pelucchi, C., et al., Smoking and other risk factors for bladder cancer in women. *Preventive medicine*, 2002. 35(2): p. 114-120.

46. Freedman, N.D., et al., Association between smoking and risk of bladder cancer among men and women. Jama, 2011. 306(7): p. 737-745.

47. Gaertner, R.R., L. Trpeski, and K.C. Johnson, A case–control study of occupational risk factors for bladder cancer in Canada. *Cancer Causes & Control*, 2004. 15(10): p. 1007-1019.

48. Shariat, S.F., et al., The effect of age and gender on bladder cancer: a critical review of the literature. *BJU international*, 2010. 105(3): p. 300.

49. Control, C.f.D. and Prevention, Self-reported influenza-like illness during the 2009 H1N1 influenza pandemic–United States, September 2009-March 2010. MMWR. *Morbidity and Mortality Weekly Report*, 2011. 60(2): p. 37-41.

50. Hewitt, M., J.H. Rowland, and R. Yancik, Cancer survivors in the United States: age, health, and disability. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 2003. 58(1): p. M82-M91.

51. Chopin, D., et al., Prognostic factors in superficial bladder cancer. *World Journal of Urology*, 1993. 11(3): p. 148-152.

52. García-Closas, M., et al., NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. *The Lancet*, 2005. 366(9486): p. 649-659.

53. Antoniou, A.C., et al., Evidence for further breast cancer susceptibility genes in addition to BRCA1 and BRCA2 in a population-based study. Genetic Epidemiology: *The Official Publication of the International Genetic Epidemiology Society*, 2001. 21(1): p. 1-18.

54. Kramer, A.A., et al., Familial aggregation of bladder cancer stratified by smoking status. *Epidemiology*, 1991: p. 145-148.

55. Lichtenstein, P., et al., Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. *New England Journal of Medicine*, 2000. 343(2): p. 78-85.

56. Olfert, S.M., S.A. Felknor, and G.L. Delclos, An updated review of the literature: risk factors for bladder cancer with focus on occupational exposures. *Southern Medical Journal*, 2006. 99(11): p. 1256-1264.

57. Ugnat, A.-M., et al., Occupational exposure to chemical and petrochemical industries and bladder cancer risk in four western Canadian provinces. *Chronic Diseases and Injuries in Canada*, 2004. 25(2): p. 7.

58. Alkubaisi, H.N.E., Incidence of cancer in Hit District. *Al-Anbar Medical Journal*, 2009. 7(1).

59. Schultzel, M., et al., Late age (85 years or older) peak incidence of bladder cancer. *The Journal of Urology*, 2008. 179(4): p. 1302-1306.

60. Steinmaus, C.M., S. Nunez, and A.H. Smith, Diet and bladder cancer: a meta-analysis of six dietary variables. *American Journal of Epidemiology*, 2000. 151(7): p. 693-702.

61. Steinmaus, C., S. Nunez, and A. Smith, Diet and bladder cancer: a meta-analysis of six dietary variables. *Alternative Medicine Review*, 2001. 6(2): p. 232-232.

62. MacKenzie, T., et al., Diabetes and risk of bladder cancer: *Evidence from a Case-Control Study in New England*. Cancer, 2011. 117(7): p. 1552-1556.

63. Vermeulen, S.H., et al., Recurrent urinary tract infection and risk of bladder cancer in the Nijmegen bladder cancer study. *British Journal of Cancer*, 2015. 112(3): p. 594-600.

64. Denzinger, S., et al., Bladder sparing approach for initial T1G3 bladder cancer: do multifocality, size of tumor or concomitant carcinoma in situ matter? A long-term analysis of 132 patients. *International Journal of Urology*, 2007. 14(11): p. 995-999.

65. Li, C.-E., et al., Chronic kidney disease as an important risk factor for tumor recurrences, progression and overall survival in primary non-muscle-invasive bladder cancer. *International Urology and Nephrology*, 2016. 48(6): p. 993-999.

66. Pasin, E., et al., Superficial bladder cancer: an update on etiology, molecular development, classification, and natural history. *Reviews in Urology*, 2008. 10(1): p. 31.

67. Gu, J. and X. Wu, Genetic susceptibility to bladder cancer risk and outcome. *Personalized Medicine*, 2011. 8(3): p. 365-374.

68. Stern, M.C., et al., Polymorphisms in DNA repair genes, smoking, and bladder cancer risk: findings from the international consortium of bladder cancer. *Cancer Research*, 2009. 69(17): p. 6857-6864.

69. Hein, D.W., Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 2002. 506: p. 65-77.

70. Rothman, N., et al., A multi-stage genome-wide association study of bladder cancer identifies multiple susceptibility loci. *Nature Genetics*, 2010. 42(11): p. 978-984.

71. Garcia-Closas, M., et al., A genome-wide association study of bladder cancer identifies a new susceptibility locus within SLC14A1, a urea transporter gene on chromosome 18q12. 3. *Human Molecular Genetics*, 2011. 20(21): p. 4282-4289.

72. La Vecchia, C. and E. Negri, Nutrition and bladder cancer. *Cancer Causes & Control*, 1996. 7(1): p. 95-100.

73. Fund, W.C.R. and A.I.f.C. Research, Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Vol. 1. 2007: *Amer Inst for Cancer Research*.

74. Kang, D., et al., Cancer incidence among pesticide applicators exposed to trifluralin in the Agricultural Health Study. *Environmental Research*, 2008. 107(2): p. 271-276.

75. Grossman, H.B. and C.P. Dinney. If cystectomy is insufficient, what is an urologist to do? in Urologic Oncology: *Seminars and Original Investigations*. 2003. Elsevier.

76. Aben, K.K., et al., Familial aggregation of urothelial cell carcinoma. *International Journal of Cancer*, 2002. 98(2): p. 274-278.

77. Lange, P.H. and D.W. Lin, Does the who and how of surgery in bladder cancer matter? Journal of clinical oncology: *Official Journal of the American Society of Clinical Oncology*, 2004. 22(14): p. 2762-2764.

78. Herr, H.W., Transurethral resection of bladder tumors, in Treatment and Management of Bladder Cancer. 2008, *CRC Press*. p. 13-22.

79. Dalbagni, G., et al., Cystectomy for bladder cancer: a contemporary series. *The Journal of Urology*, 2001. 165(4): p. 1111-1116.

80. See, W.A. Postoperative nomogram predicting risk of recurrence after radical cystectomy for bladder cancer: International Bladder Cancer Nomogram Consortium, Bochner BH, Kattan MW, Vora KC, Department of Urology, Memorial Sloan-Kettering Cancer Center, Kimmel Center for Prostate and Urologic Tumors, New York, NY. in Urologic Oncology: *Seminars and Original Investigations*. 2007. Elsevier.

81. Daneshmand, S., Ahmadi, H., Schuckman, A. K., Mitra, A. P., Cai, J., Miranda, G., & Djaladat, H. (2014). Enhanced recovery protocol after radical cystectomy for bladder cancer. *The Journal of Urology*, 192(1), 50-56.

82. Biot, C., et al., Preexisting BCG-specific T cells improve intravesical immunotherapy for bladder cancer. *Science Translational Medicine*, 2012. 4(137): p. 137ra72-137ra72.

83. Lamm, D.L., et al., Maintenance bacillus Calmette-Guerin immunotherapy for recurrent TA, T1 and carcinoma in situ transitional cell carcinoma of the bladder: a randomized Southwest Oncology Group Study. *The Journal of Urology*, 2000. 163(4): p. 1124-1129.

84. Rosenberg, J.E., P.R. Carroll, and E.J. Small, Update on chemotherapy for advanced bladder cancer. *The Journal of Urology*, 2005. 174(1): p. 14-20.

85. Boyle, H., A. Fléchon, and J. Droz, Treatment of uncommon malignant tumours of the bladder. *Current Opinion in Urology*, 2011. 21(5): p. 309-314.

86. Vale, C., Neoadjuvant chemotherapy in invasive bladder cancer: update of a systematic review and meta-analysis of individual patient data: advanced bladder cancer (ABC) meta-analysis collaboration. *European urology*, 2005. 48(2): p. 202-206.

86. Yu, E. Y. W., Wesselius, A., Mehrkanoon, S., Goosens, M., Brinkman, M., van den Brandt, P., & Zeegers, M. P. (2021). Vegetable intake and the risk of bladder cancer in the BLadder Cancer Epidemiology and Nutritional Determinants (BLEND) international study. *BMC medicine*, 19(1), 1-15.

87. Bates, M. N., Smith, A. H., & Cantor, K. P. (1995). Case-control study of bladder cancer and arsenic in drinking water. *American Journal of Epidemiology*, 141(6), 523-530.

88. Tracey, E., Roder, D., Luke, C., & Bishop, J. (2009). Bladder cancer survivals in New South Wales, Australia: why do women have poorer survival than men?. *BJU International*, 104(4), 498-504.

89. Hartge, P., Harvey, E. B., Linehan, W. M., Silverman, D. T., Sullivan, J. W., Hoover, R. N., & Fraumeni, J. F. (1990). Unexplained excess risk of bladder cancer in men. JNCI: *Journal of the National Cancer Institute*, 82(20), 1636-1640.

90. Shariat, S. F., Milowsky, M., & Droller, M. J. (2009, November). Bladder cancer in the elderly. *In Urologic Oncology: Seminars and Original Investigations* (Vol. 27, No. 6, pp. 653-667). Elsevier.

91. Froehner, M., Brausi, M. A., Herr, H. W., Muto, G., & Studer, U. E. (2009). Complications following radical cystectomy for bladder cancer in the elderly. *European urology*, 56(3), 443-454.

Appendix-A

Questionnaire for the Bladder Cancer Research

Purpose: The purpose of this study is to investigate the most prevalent cause of bladder cancer in Pakistani population. (Performa Reference: American Association for Cancer Research).

Date.....

Patient's Details:

Patient Name:.....

Sex:.....

Age: .....

Weight: .....

Height:.....

Area:.....

- **Family History**

1. How long you have been suffering from bladder cancer?

4 years	3 year	So long

2. Are your family members suffering from Bladder cancer?

Yes	No

3. Which family member is suffering from the Bladder cancer?

Mother	Father	Brother/Sister	Grand Parents

• Dietary History

1. Major portion of your diet consist of?

Vegetables	Meat	Fluid	Mix

2. For how long time you are smoking?

4 year	5 year	6 year	More

3. Are you taking Alcohol or any other fluid?

Yes	No	Not Noticed

4. Are you employed?

Yes	No

5. What field you are employed in?

6. Do you have some exposure with arsenic?

Yes	No

7. How many cigarettes you are inhaling per day?

• Medical History:

1. Have you suffering from Cancer of lower urinary tract? ☐ Yes ☐ No ☐ never noticed ☐ test not done.

2. Have you gone through any abdominal surgery? ☐ Yes ☐ No

3. Do you have any type of addiction? ☐ Yes ☐ No

4. Are you taking medication for any Medical issue? ☐ Yes ☐ No

5. If yes, for how long? .....

6. How long after the illness consulted to Doctor? 1 year ☐ 2 years ☐

3 years ☐ 4 years ☐

7. Your disease is self-diagnosed?

Yes ☐ No ☐

8. What is Histopathology Type of cancer?

Carcinoma Sarcomas Adenocarcinoma Lymphomas Small cell carcinomas Transitional cell carcinoma

9. What is MRI Result?.....

10. What is ultrasound report of patient?.....

11. Does patient cancer spread to other parts of the body?

Metastasis organ.....