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Detection of Gene Mutation Associated with Bladder Cancer through DNA Polymorphism

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ABSTRACT

The periodic statistic defines encyclopaedically, urothelial bladder melanoma ranks the fifth most common cancer in men and the fifteenth in women, and the prevalence rate increases in people aged than 60 (1). Oncologists and pathologists in Egypt have suggested that Egypt has one of the loftiest prevalence rates of bladder cancer among men in the world, estimated prevalence rate of37.7 cases per 100,000 males (2). The histopathological profile of bladder cancer has changed significantly over the once 26 times. Historically, scaled cell melanoma but now a days, transitional cell melanoma has come the most frequent type and this shows the change in the epidemiology of the complaint (3). Individual variation in the inheritable constitution of humans may affect the host responses to constant assaults from exogenous and endogenous carcinogens, which will ultimately impact cancer threat, complaint prognostic and clinical outgrowth. Then we're agitating about a number of associations and limitations of current inheritable vulnerability exploration and suggest unborn directions in molecular epidemiology study. DNA polymorphism, a condition in which one of two different but normal nucleotide sequences can live at a particular point in DNA, the appearance of heritable mutation in a population at high frequency. Since heritable mutations eventually lead to elaboration, hence study of DNA polymorphism is important from the evolutionary perspective for the progression of bladder cancer. Different alleles of a gene produce different phenotypes which can be detected by making crosses between parents with different alleles of two or further genes. Also, by determining recombinants in the get, a inheritable chart can be derived. DNA polymorphisms constitute molecularly defined differences between individual mortal beings; hence the dominant homozygous, heterozygous and sheepish homozygous alleles can be helpful for the identification of heritable bladder cancer normal, carrier and diseased independently. The present study aimed to find out the association of polymorphic variations in the glutathione S transferase gene (GSTP1) with the bladder cancer in an Eastern Indian Population. From worldwide and civil studies, it was hypothecated that the GSTP1 gene polymorphism is a common event in people suffering from bladder cancer. It was hypothecated that the PCR- RFLP fashion is a good and robust fashion for discovery of this polymorphism. Consequently, the present study was designed and executed.

Keywords: RFLP- PCR, bladder cancer, DNA polymorphism, GSTP1 polymorphism, genomic differences, DNA, cancer.

Literature Review

Lately, the RFLP- PCR fashion has been used for the discovery of the genomic differences (4) in mortal cancers, revealing that genomic insecurity passed constantly in utmost of renal excrescences, brain excrescences, lung cancer, bone cancer, colon cancer, head and neck scaled cell melanoma, acute lymphoblastic leukaemia, hepato cellular melanoma, skin cancers and pituitary adenomas. It plays a significant part in the discovery of bladder cancer and progression. Bladder cancer (BCa) is the most common malice of the urinary tract and one of the most current cancers worldwide. While the clinical approach to BCa has remained largely unchanged for numerous times, recent discoveries have paved the way to a new period of opinion and operation of the complaint. BCa-specific mortality started to drop in the regions with a wide range of conditioning leading to lesser social mindfulness of the threat factors and the decline in carcinogenic exposure. The urologic community refines the part of transurethral surgery towards further rigorous and high-quality ways. New agents have been approved for cases with BCG failure who faced radical cystectomy so far. Although radical junking of the bladder is the gold standard for muscle invasive cancer operation, the extent and clinical value of lymphadenectomy is presently heavily challenged in randomized trials. likewise, druthers to perioperative chemotherapy have arisen to increase the liability of complete treatment delivery and successful oncological issues. Eventually, advancements in molecular biology and our understanding of tumorigenesis open the period of individualized drug in bladder cancer. In the present review, the status and unborn directions in bladder cancer epidemiology, opinion and operation are completely bandied.

Research hypothesis

The RFLP-PCR method has the potential to detect a wide range of genomic alterations, hence a suitable way to detect bladder genetic polymorphisms and bladder cancer risk and disease progression.

Aim

The aim of this study is to examine the possible application of Restriction fragment length polymorphism (RFLP), technique after the polymerase chain reaction (PCR) method, as a genetic test for identifying the significant genomic alterations in bladder cancer patients.[1]

Materials required

Patients and controls

The study was prospective and included 100 male patients admitted at Calcutta National Medical Hospital. Their age ranged from 30-80 years. We used the hospital information system and medical record to collect data about these patients and their tumors.

Polymerase chain reaction (PCR):

(A)A thermostable DNA polymerase: For routine PCR it is obtained from the bacillus Thermus

aquaticus, a member of the thermophilic archaebacteria. Its optimum temperature of activity is 75 to 80-degree C. it is added in the reaction mixture at a concentration of 0.5 to 2.5 IU.

(B) Oligonucleotide primers: Two oligonucleotide primers with opposite complementarity for the flanking sequence of the gene are needed. Typically, 0.1 to 0.5 uM of each primer is added to the final reaction mixture (total amount being 5 to 25 pmol). Oligonucleotide primers should have following characteristics:

i) They should not be self-annealing,

i) They must be specific for the gene targeted,

ii) They should not form any secondary structure,

iv) Total GC content should be within 40 to 60 percent and similar to that of the amplified product.

v) All four bases should be evenly distributed throughout the length of the primer.

vi) Primers should be 18 to 25 bp in length. The number of forward and reverse primers must not differ by more than 3 base pairs.

vii) no inverted repeat sequence or self-complementary sequences should be present for more than 3 bp length to prevent hairpin loops.

vii) Melting temperature (Tm value): Tm value of the primers should not differ by more than 5 degrees C. The Tm value of the amplified products should not differ from the Tm value of the primers by more than 10-degree C.

(C) Deoxynucleotide triphosphates (dNTPs): dNTPs are added at a concentration of 200 -250 uM for the total PCR reaction mixture. dNTPs are stable at alkaline pH. Hence, the pH of PCR buffer is also made to be alkaline.

(D) Divalent cations: Magnesium is the commonest divalent used most effectively. It is used at a concentration of 1.5 to 6 mM under different conditions and dNTP concentration. The concentration of magnesium ion thus should be titrated accordingly.

(E) Template DNA: The template DNA should be free of excess phenol, EDTA and proteinase K. Up to 1 ug of template DNA can be added for mammalian gene PCR. For the yeast, bacterial and plasmid DNA the amount is 10 ng. 1ng. and 1pg respectively

(F) Autoclaved buffered solution: 10 mM of tris-Cl with a pH of 8.3 to 8.8 is added. During heating process, the pH drops to 7.3 at 72 degrees centigrade. So, maintenance of buffer pH above 8 is necessary.

Methodology:

The Phenol-Chloroform DNA extraction method is one of the most useful and commonly used methods that yields high molecular weight DNA and therefore can be utilized in situations where PCR typing is performed. It is the preferred method of extraction for samples that are old or degraded because it consistently yields higher quantity of DNA [5]. There is a usefulness of this DNA fingerprinting method for the detection of genomic instability in bladder cancer

Restriction fragment length polymorphism (RFLP), is a PCR based fingerprinting technique that amplifies random DNA fragments. In PCR, the primers anneal to complementary sequences at different genomic loci and amplify the target sequence. Then the process is followed by RFLP where the restriction enzyme has been used is mutation specific. So during the gel run only the mutated DNA is fragmented by the restriction enzyme, which is determined by the generated fragment length.

1. Sample collection and processing:

DNAs were extracted from the exfoliated cells in the urine of 100 male bladder cancer patients, using the Phenol-Chloroform DNA extraction method, and the yielded DNAs were amplified with the random primer. The amplified PCR products from RFLP analysis were electrophoretically separated in

agarose gels; banding profiles were visualized by ethidium bromide staining and demonstrated under ultraviolet light. The genomic alterations were clearly apparent in tumor RFLP-PCR patterns, by the loss of normal bands and the appearance of new tumor-related bands, as compared to the controls. Our study suggests the potential of RFLP-PCR method to produce diagnostic markers for analysing the genomic instabilities in bladder tumors.

Urine samples were collected from total 100 healthy individuals and bladder cancer patients.

Samples were centrifuged at 1500xg for 15 min to pellet the exfoliated cellular material. In patients with haematuria, the cell pellet was resuspended with 400 ul red blood cell lysis buffer.[6]A dry pellet containing the cells was transferred into an Eppendorf tube (1.5 ml) and centrifuged for 7 min at 10,000xg (4 °C) [7][8].

2. Phenol-chloroform DNA extraction

Digestion buffer (500 µl) was added to the dry pellet, and the tube was gently inverted several times to resuspend the pellet. Proteinase K (50 µl of 20 mg/ml) was added, and then the tube was incubated in a water bath at 56 °C overnight. An equal volume of Phenol: Chloroform: Isoamyl alcohol solution (25:24:1) was added to the suspension and mixed gently by inverting the tube for a few minutes. Samples were centrifuged at 13,200xg (4 °C) for 15 min and the upper aqueous layer was transferred to a new sterile Eppendorf [7]. RNase (10 µl of 10 mg/ml) was added and kept for incubation at 37 °C for 4 hours. An equal volume of Phenol: Chloroform: Isoamyl alcohol solution was added, and the tube was centrifuged again at 10,000xg (4 °C) for 10 min. The upper portion of the aqueous layer was shifted into a sterilized fresh microcentrifuge tube. 1 M sodium chloride solution (NaCl) was added and mixed well, then six-tenth volume of chilled isopropanol [8].

DAY 1

1.1ml of whole blood is taken in falcon tube containing EDTA, works as a chelating agent in the DNA extraction. It chelates the metal ion present in the enzymes and as we all know that the metal ions and the co factor which increases the activity of the enzyme. By chelating the metal ions, it deactivates the enzyme therefore reduces the activity of DNase and RNase.

2.Addition of 1.5 ml of cell lysis buffer is added to the mixture.

3.Centrifuging at 10000 rpm for 15 mins at 4-degree C. The supernatant is separated out which contains water, dissolved proteins, lipoproteins.

4.500 micro ml of nuclear lysis buffer + 10 micro ml proteinase K is added of concentration 20mg/ml.

5.Incubated in 37 degree C, water bath, overnight, so the proteins, suspend lipids, digest

Other materials to make DNA free.

DAY 2

1. Then we have added 1ml of TRIS saturated phenol to viscous homogenous mixture of blood and other reagents added.

2.Then we centrifuged it at 10,000 rpm, for 7 mins at 4- degree C.

3. Then the pellet (phenol + impurities) was separated out.

4.And extraction of supernatant (containing of water +lipid+ nucleic acid).

5.Then we added Chloroform +isoamyl alcohol (24:1)

6.Then we centrifuged at 10,000 rpm ,7 mins,4- degree C.

7.The pellet was separated out.

8.Supernatend was extracted and put in different Eppendorf.

9.Then we added 10 micro ml ,3 M sodium acetate.

10. 1.5 mL, ice chilled 95% ethanol was added.

11.Then we centrifuged it at 10,000 rpm,5mins,4 -degree C.

12.Supernatand was extracted and pellet retained.

13.50 micro l, TE (Tris EDTA) buffer (pH 7.4) added to solubilize the pellet.

14.finally we microfuged at 4000 rpm ,2mins.

3. PCR

(i) PCR Master mix: The master mix provided here contains the alkaline Tris buffer, dNTPs, Mg and Taq polymerase. The master mix provided here is 2x I; e it has to be diluted twice in the final mixture volume. For a final mixture of 25 ul we add 12.5 ul of the PCR master mix.

(ii) Forward primer: Stock primer is diluted with nuclease free water or tris buffer as recommended for that particular primer criteria. From this dilute solution 1 ul is added to get the desired concentration (refer to the primer section of this protocol; here we get a final amount of 10 pmol by adding 1ul of this primer).

(iii) Reverse primer: Same as the forward primer,

(iv) Template DNA: 1ul (total 0.5 to 1 ug)

(v) Sterilized autoclaved nuclease free water: 9.5 ul

ALL PREPARATIONS ARE MADE ON ICE.

(vi) PCR run: For this PCR for the GSTP1 rs 1695 the following PCR protocol is adopted.

a) Initial denaturation step :95 degree C for 2 minutes: single step

For 30 cycles:

b) Denaturation at 95 degree C for 30 seconds

c) Annealing at 60 degree C for 30 seconds

d) Extension (replication) at 72 degree C for 1 minute

e) Final extension phase at 72 degree C for 5 minutes: single step

f) Storage phase at 4 degree C till further use.

After completion of the PCR the PCR amplicon is run through 2% agarose gel against known 100 base pair DNA ladder. For visualization of the separated DNA strands, ethidium bromide is mixed in the gel at the concentration of 0.5 ug/ml of gel (from a 10 mg/ml of stock solution).

PHASE I: Digestion of the PCR product by a suitable restriction enzyme to detect the target

mutation. Restriction digestion of the PCR product is done using the following protocol for the total volume of 30 ul as follows:

i) PCR product: 10 ul,

ii) 10x buffer: 2 ul

ii) Alw261 (BsmAl): 2 ul

iv) Deionized nuclease free water: 16 ul

Total volume is 30 ul.

The mixture is incubated for 2 hours at 37 degree C. The digested product is run on 3% agarose gel for detection of digested bands against DNA ladder of known base pair numbers. If the products are needed to be stored, then the restriction enzymes must be inactivated by heating the mixture for 20 minutes at 80 degrees centigrade. Final interpretation is done according to the number and position of the bands present in the gel.

PCR protocol and programme:

1. Denaturation: This process is needed to separate the two strands of DNA for primer attachment. The temperature for denaturation depends on mainly two factors:

i) The GC content and ii) The length of the DNA. Generally, the Taq polymerase can withstand the temperature of 94 to 95 degree C for about 30 cycles. So, routinely this temperature is used for about one minute most frequently for those genes with a GC base pair of about 54 to 55 percent. However, in the initial step, the denaturation is done for a longer period of 4 to 5 minutes to enable the longer DNA strands to denature.

2. Annealing of the primers to the template DNA: Annealing temperature should not be too high or too low. At too high temperatures the primers poorly pair up with their cognate template strands resulting in poor yield of the PCR product. At too low temperature, the binging becomes so frequent that it becomes non-specific. Generally, the template temperature is kept 3-5 degree C lower than the melting temperature prescribe at which the primers get separated from their cognate DNA strands.

3. Extension of the oligonucleotide primers: After the binding of the primers with their template strands, the polymerization is carried out at about 72 to 78 degree C which is the optimal temperature for the Taq polymerase enzyme. At this temperature, the DNA Taq polymerase adds about 2000 nucleotides per minute and for 1000 bp product size 1 minute time is sufficient.

4. Final extension time: Generally, this is about three times of the polymerization time and is used after completion of the cycles to complete all remaining polymerization reactions.

5. Number of cycles: Number of copies depend upon the initial concentration of the template DNA and the efficiency of the Taq polymerase enzyme and the primers. For the human genes, at least 25 cycles are needed to produce acceptable levels of amplified products for a single copy target sequence. For optimum production of the amplified sequence for the human genes ideally 30 cycles are provided that produce about 10 copies of the target sequence.

Based on these basic principles of PCR,

(1) Amplification of our target gene by the PCR technique,

(2) Digestion of the PCR product by suitable restriction enzyme to detect the target mutation.

(3) Amplification of the target gene by PCR

Here re we are amplifying the GSTP1 gene followed by its digestion. GSTP1 stands for the pi 1 isoform of the glutathione S transferase enzyme that is involved in the detoxification of various xenobiotics including the carcinogenic substances. People with an SNP of rs 1695 A>G) that results in replacement of lleu by Val at the 105h position of the protein has a reduced capability of detoxifying carcinogenic substances that predispose them to various types of cancer. So, people with this SNP nave a more lifetime risk of cancers of various organs including prostate, urinary bladder, kidney, breast etc. In the present protocol we will perform the PCR amplification of this gene with its restriction digestion to find out whether this particular SNP is present in our sample or not.

4. Restriction fragment length polymorphism (RFLP)

Glutathione-S-Transferases (GSTs) belongs to tumor suppressor proteins which restrict the initiation and progression of tumor genesis by detoxifying different toxic carcinogens and reactive oxygen species (ROS). The *GSTP1* gene is approximately 4 kb in length, comprises 7 exons and 6 introns and codes for a 715 bases mRNA [9]. GSTs have several isozymes with almost similar functions in different tissues. They are responsible for metabolism and biosynthesis of various metabolites including detoxification of exogenous carcinogen chemicals like polycyclic aromatic hydrocarbon which are abundant in diesel fuels, cigarette smoke and grilled meats [10]. These detoxify several carcinogenic xenobiotics by conjugation through glutathione during the phase II, process of detoxification of the electrophonic carcinogenic compounds. Specific GST isoforms in the (M1), (T1) and (P1) classes are highly expressed in the prostate tissues. Among the large family of their isoenzymes, the P1 class of enzyme GSTP1-1 is well studied in different types of cancers. Expression of *GSTP1- 1* has been identified to be associated with cytotoxic drug resistance. The potential *GSTP1* gene promoter site remains unmethylated and an adenine (A) at the 303 positions. Another important single nucleotide polymorphism (SNP) in the *GSTP1* gene was found to be Ile105Val (rs1695 *A* > *G*) that replaced valine by isoleucine at the 105thpostion of the GSTP1 protein causing significant reduction in the detoxifying capability of this important GST isoenzyme. However, in line with all polymorphic studies, outcomes of different studies reporting this SNP have been contradicting for prostate cancer risk varying indifferent regions of the world significantly.

Restriction digestion

For PCR we used the PCR master mix from Thermofisher, USA. The forward and reverse primers were *5´GTCT*CT*CATCCTTCCACGCA*3´.and*5´CTGCACCCTGACCCAAGAA*-3´ respectively. PCR products obtained were digested using the restriction enzyme Alw261 obtained from Thermofisher USA. Restriction digestion products were identified on 3% agarose gel against 100 bp DNA ladder using the gel doc system.

ISUP Grading

The grading system proposed by the International Society of Urological Pathology (ISUP) have improved the overall Gleason grading system. Accordingly, the prostate cancer patients in our study were divided into five distinct ISUP grades; Grade 1: Gleason's score ≤6, Grade 2: Gleason's score 3 + 4 = 7, Grade 3: Gleason's score 4+3 = 7, Grade 4: Gleason's score 4+4 = 8, and Grade 5: Gleason's score 9 and 10[11], recommended by National

Comprehensive Cancer Network [12].

Gleason score bladder cancer

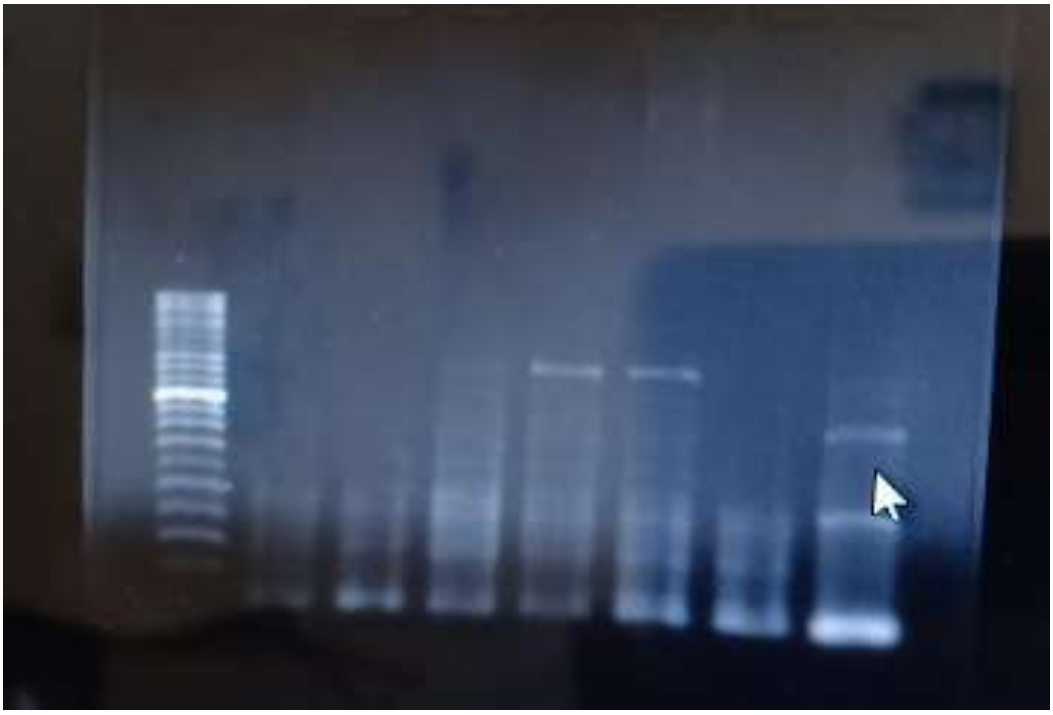
Gleason score 7 indicates moderately aggressive cancer. Cancers with Gleason scores of 8 to 10 are highly aggressive. The prostate gland is typically the size of a walnut, located below the bladder and in front of the rectum in men [13].

Statistical Analysis

The distribution of different *GSTP1* allelic comparison between the case and the controls was detected by chi square test process and analysis of odds ratio. Statistical comparison of ISUP grade distribution between the Ile/Ile, Ile/Val and Val/Val was done by post hoc ANOVA with

Bonferroni correction. Dependence of severity of bladder cancer as indicated by ISUP grading was done by logistic regression analysis. All statistical analyses were done using SPSS software version 20 for windows [14].

Result



Lane no: 1st 2nd 3rd 4th 5th 6th 7th 8th

Fig 1: In the picture, the gel slab is placed in an UV Transilluminator and the capture in laptop. 1st lane is the ladder sample of 50 bp. The 5th and the 6th lane clearly showed band at approximately 650 bp, hence recessive homozygous disease.8th lane the band reflects the cut homozygote with two bands at 400 and 250 bp region.

Genomic DNAs were extracted, purified from all studied urine samples and the un purified DNAs were excluded. Following the steps of DNA extraction, samples were run in 0.7% agarose gels. The genomic DNAs from urine samples of bladder cancer patients were isolated.

In our previous work, we applied RFLP- PCR. In addition, the RFLP-PCR profiles of all patients and controls were generated by the same synthetic primer. These DNA fragments can be used as diagnostic markers for this histologic type. We need to mention that initially we amplified the DNA sample by PCR that we extracted then we run it on the gel slap and the DNA went through digestion.

1st lane is loaded with control sample.

And the 5th and the 6th lane clearly showed band at approximately 650 bp after 2/3 gel run.

, hence recessive homozygous allele that is detected as bladder cancer disease.

8th lane showed a completely cut homozygote showing two bands at 400 and 250 bp regions.

The heterozygous allele indicated carrier person, showed more than one band digestion.

The recessive homozygous allele showed the bladder cancer affected person, showed one clear band of DNA digestion. Bladder cancer are mostly inherited, so by restriction digestion it can be detected and hence can be treated early. Those showed one clear digestion are detected as bladder cancer patients.

Hence, it can be done reliably by RFLP -PCR method.

Discussion

Expression of *GSTP1* is regulated mainly at the transcriptional level. That has been suggested that replacement of isoleucine with a comparatively less hydrophilic and less bulkier valine addition in the protein results in the alteration effects in the capacity of the substrate binding to its catalytic site resulting the reduction in detoxifying capability of the prooxidant heterocyclic amine carcinogens [15]. Loss of detoxifying capability due to mutant *GSTP1* gene has been reflected by a direct association between the lipid peroxidation product 4-hydroxynonenal in some recent studies [16]. Results of our study not only support this view but in addition the one way and post hoc ANOVA suggest that the cancer patients having the *G* alleles have more progressive form of the disease as evident from their higher ISUP grade. To find out the contribution of the mutant G allele on the ISUP grades in prostate cancer patients, we carried out logistic regression analysis considering the ISUP grade as a dependent variable on both A and G alleles of all three

genotypes. Results showed a significant predictive role of only G alleles on the ISUP score. Polymorphic changes from A to G not only alter the substrate binding property of this enzyme, but also alter its signal transduction process related to the regulation of cell growth. *GSTP1* enzyme protein is also closely linked to the signal transduction process involving Jun N terminal kinase (JNK pathway) [17]. Moreover, its specific inhibitor TER 199 has been found to stimulate the growth of granulocytes [18] suggesting an. inhibitory effect of *GSTP1* mediated intracellular signal transduction pathway on abnormal cell growth. These cellular mechanisms provide plausible biochemical explanations for the inducing effects of Ile105Val SNP (313 A to G) on carcinogenesis and its progression to more severe outcome.

RFLP-PCR technique has been successfully used in the present project to detect the polymorphic variations of this polymorphism. The results of the RFLP were confirmed in comparison to the direct sequencing of the 10% of the PCR products.

The major limitation of the present study is a relatively modest number of sample size of cancer patients that could be included in the study according to the stipulated inclusion

and exclusion criteria. Hence, based on our statistical calculations and result output we suggest that *GSTP1* polymorphism at Ile105Val level should be explored in much wider areas involving different regions so that it can be considered as a prognostic indicator for bladder cancer in those study populations. Assessment of this polymorphism at the earlier stages of the disease may also help in planning more aggressive management of the disease for preventing further spread and its lethal outcome.

Future aspects

The detection we preferred are reliable and we will like to further continue research for early detection and reduction of slow progression and the treatment process as well in the most suitable way [19].

• About Bladder Cancer

• Causes, threat Factors, and Prevention

• Early Discovery, opinion, and Carrying

• Treatment

When you ’re told you have bladder cancer

• What type of bladder cancer can have?

• Do you suppose the cancer has spread beyond bladder?

• What's the stage and grade of the cancer, and what does that mean?

• Will I need any other tests before decide on treatment?

• Do any affiliated complaint can show up?

• The discovery through RFLP PCR is dependable or time saving for the opinion of the complaint?

When deciding on a treatment plan

• What are treatment options?

• What's the thing of each treatment?

• Any percentage prediction of the chances to cure?

• If the bladder is removed, what are my options for passing urine? What are the pros and cons of each?

• How soon do need to start the treatment?

• How long will treatment last? What will it be like? Where will it be done?

• What pitfalls or side goods should be anticipated? How long are they likely to last?

• Will treatment affect diurnal conditioning?

• How likely is it that the cancer will come back? Is there anything to help lower this threat?

• What will we do if the treatment doesn’t work or if the cancer comes back?

During treatment

• What are the signs that the treatment is working?

• Is there anything to help manage side goods?

• Are there any limits on what one can do?

• A internal health professional if one launch to feel overwhelmed, depressed, or worried?

After treatment

• What signs and symptoms should one watch for?

• What type of follow- up will one need after treatment?

• How frequently will one need to have follow- up examinations and tests?

• How will one know if the cancer has come back? What should one watch for?

• What would be the options be if the cancer does come back?

Conclusion

Bladder cancer (BC) has the loftiest continuance treatment costs per case of all cancers (20) (21). The high rush rate and ongoing invasive monitoring demand are the crucial contributors to the profitable and mortal risk of this complaint (22). The purpose of this paper was to use the recent literature to identify openings to know the better ways for opinion of Bladder cancer and possible ways to apprehensive, help as important as possible in future.

References

1. Guey, L. T., García-Closas, M., Murta-Nascimento, C., Lloreta, J., Palencia, L., Kogevinas, M., ... & EPICURO/Spanish Bladder Cancer Study investigators. (2010). Genetic susceptibility to distinct bladder cancer subphenotypes. *European urology*, *57*(2), 283-292.

2. El-Far, M., Abol-Enein, H., Zakaria, A., & El-Gedamy, M. Application of random amplified polymorphic DNA-PCR technique in early diagnosis of bladder cancer using urine samples.

3. Felix, A. S., Soliman, A. S., Khaled, H., Zaghloul, M. S., Banerjee, M., El-Baradie, M., ... & Wilson, M. L. (2008). The changing patterns of bladder cancer in Egypt over the past 26 years. *Cancer Causes & Control*, *19*(4), 421-429.

4. Faddagh, M. S., Hussain, N. A., & Al-Badran, A. I. (2012). DNA fingnerprinting of eight cyprinid fish species of Iraqi inland waters using RAPD-PCR technique. *Advances in Life Sciences*, *2*(2), 9-16.

5. Butler, J. M. (2005). *Forensic DNA typing: biology, technology, and genetics of STR markers*. Elsevier.

6. Maniatis, T. (1982). Molecular Colning. *A laboratory manual*.

7. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., & Struhl, K. (1992). Short protocols in molecular biology. *New York*, *275*, 28764-28773.

8.El-Far, M., Abol-Enein, H., Zakaria, A., & El-Gedamy, M. (2013). Detection of genomic instability in renal cancer by random amplified polymorphic DNA analysis from urine sample as a non-invasive method: Potential use in diagnosis. *Cancer Sci Res Open Access*, *1*(1), 7.

9. Roy, S., Dasgupta, A., Chatterji, S., & Karmakar, D. (2019). Correlation of GSTP1 Polymorphism with Severity of Prostate Cancer in an Eastern Indian Population. *Emerging Research in Medical Sciences Vol. 1*, 118-127.

10. Knudson Jr, A. G. (1986). Genetics of human cancer. *Annual review of genetics*, *20*(1), 231-251.

11. Pierorazio, P. M., Walsh, P. C., Partin, A. W., & Epstein, J. I. (2013). Prognostic G leason grade grouping: data based on the modified G leason scoring system. *BJU international*, *111*(5), 753-760.

12. Abdollah, F., Sun, M., Suardi, N., Gallina, A., Capitanio, U., Bianchi, M., ... & Briganti, A. (2012). National Comprehensive Cancer Network practice guidelines 2011: Need for more accurate recommendations for pelvic lymph node dissection in prostate cancer. *The Journal of urology*, *188*(2), 423-428.

13. Kamat, A. M., Hahn, N. M., Efstathiou, J. A., Lerner, S. P., Malmström, P. U., Choi, W., ... & Kassouf, W. (2016). Bladder cancer. *The Lancet*, *388*(10061), 2796-2810.

14. Ardelt, P., & Böhle, A. (2002). Molecular aspects of bladder cancer IV: gene therapy of bladder cancer. *European urology*, *41*(4), 372-381.

15. Sundberg, K., Johansson, A. S., Stenberg, G., Widersten, M., Seidel, A., Mannervik, B., & Jernström, B. (1998). Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. *Carcinogenesis*, *19*(3), 433-436.

16. Song, X., Wang, R., Xiao, F., Zhang, S. X., Gong, M., Wang, X. L., ... & Huang, J. Y. (2017). Expressions of glutathione S-transferase P1 and 4-hydroxynonenal and the progression of prostate cancer. *Zhonghua nan ke xue= National Journal of Andrology*, *23*(5), 412-416.

17. Adler, V., Yin, Z., Fuchs, S. Y., Benezra, M., Rosario, L., Tew, K. D., ... & Ronai, Z. E. (1999). Regulation of JNK signaling by GSTp. *The EMBO journal*, *18*(5), 1321-1334.

18. Kauvar, L. M., Morgan, A. S., Sanderson, P. E., & Henner, W. D. (1998). Glutathione based approaches to improving cancer treatment. *Chemico-biological interactions*, *111*, 225-238.

19. Dobruch, J., & Oszczudłowski, M. (2021). Bladder cancer: Current challenges and future directions. *Medicina*, *57*(8), 749.

20. Ferlay, J. F. (2001). GLOBOCAN 2000. Cancer incidence, mortality and prevalence worldwide, version 1.0. *IARC cancerbase*.

21. Miller, B. A., & Kolonel, L. N. (1996). *Racial/ethnic patterns of cancer in the United States, 1988-1992* (No. 96). US Department of Health and Human Services, National Institutes of Health.

22. Sievert, K. D., Amend, B., Nagele, U., Schilling, D., Bedke, J., Horstmann, M., ... & Stenzl, A. (2009). Economic aspects of bladder cancer: what are the benefits and costs? *World journal of urology*, *27*(3), 295-300.