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Research Article

Impact of Genetic Polymorphisms of MDR1 Gene in the Chemotherapeutic Treatment of Cervical Cancer: A Case Control Study

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ABSTRACT
Polymorphisms in genes coding for metabolising enzymes can affect drug efficacy and toxicity. The cervical cancer is the leading cause of death from gynecological malignancy, and the second most commonly diagnosed gynecologic malignancy Aim and objective: The present study will be undertaken with an objective to evaluate the clinical significance of polymorphisms in drug-metabolising enzymes in cervical cancer patients. Materials and methods: Genomic DNA isolation: Genomic DNA isolation will done for control blood samples and homogenised tumor samples by salting out method followed by ethonol extraction. Agarose gel elctrophorosis: presence of DNA well cheked by using 0.8% agarose gel electrophorosis. Polymarase chaine reaction (PCR): PCR amplification will be done by using specific set of primers and PCR condition followed by agarose gel electrophoresis to conform the amplified product, Single strand conformatory polymorphisms: SSCP of the PCR product will done for detecting the presence of polymorhisms in the amplifide exons from cancer samples compared to helthy samples, Sequenceing: Sequence analysis will done for the strands which showing shifts in SSCP. Results: Detection of single nucliotide polymorphisms in Cervical cancer patients lead to safer chemotherapetic procedures for their treatment and play role in Disease prognosis, relative risk individually in developing cervical cancer. Conclusion: Hence this study form the basis of an effort to reduece the trama and suffering of the cervical cancer patient.

Key words: MDR1, PCR, Polymorphism, Cervical cancer, toxicity

INTRODUCTION

The MDR1 multi drug resistant gene, one of the better characterized drug transporters that play an important role in protecting the body against xenobiotic substances and toxic compound. P-Glycoprotein (Pgp) was linked to multidrug resistance (MDR) in mammalian cell lines and human cancers, P-glycoprotein (P-gp) is the Trans membrane multi drug transporter encoded by the MDR1 gene. The tissue localization of Pgp suggests that the protein plays a physiological role in the protection of susceptible organs like the brain, testis and inner ear from toxic xenobiotics, the secretion of metabolites and xenobiotics into bile, urine, and the lumen of the gastrointestinal tract, and possibly the transport of hormones from the adrenal gland and the uterine epithelium.

Pgp has the ability to interact with literally hundreds of structurally diverse substrates which are generally nonpolar, weakly amphipathic compounds, and include natural products, anticancer drugs, steroids, fluorescent dyes, linear and cyclic peptides, ionophores, etc.

Role of P-glycoprotein in drug therapy

Pgp substrates are used in the treatment of common human diseases. The protein plays a key role in drug absorption. Pgp is an important determinant in the pharmacokinetic profile of many drugs, and their clinical response. Pgp substrates include anti-cancer drugs, HIV protease inhibitors. Modulation of P-glycoprotein in cancer treatment mainly. The failure of chemotherapy treatment to cure human cancers is the ability of tumor cells to become simultaneously resistant to several different anti-cancer drugs. Many mechanisms are known to contribute to MDR in tumor cells, of which the presence of multidrug efflux pumps is only one. There are three ABC family members of MDR are Pgp, MRP1 (ABCC1) and BCRP (ABCG2), likely to be the major drug efflux pumps expressed in human cancers. There is still no consensus on the useful of MDR modulators in treating human cancers, and the controversy is likely to continue. There are multiple interacting pathways for activation of MDR1. Most MDR1 transcripts arise from downstream promoter sequences located in the middle of exon 1, which lacks a TATA box. An inverted CCAAT box interacts with the trimeric transcription factor NF-Y, and the Sp family transcription factors, Sp1 and Sp3. MDR1 transcription is

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upregulated as part of exposure to anticancer drugs and carcinogens.

P-glycoprotein gene polymorphisms during drug therapy

The first polymorphism to be reported in the human MDR1 gene was the G2677T variant, which results in the amino acid change A893S. Later 30 single nucleotide polymorphisms (SNPs) have been discovered by sequencing the MDR1 gene in large numbers of individuals of different ethnic Origin.

Genetic Polymorphisms of MDR1

MDR1, located on chromosome 7q21.1, is composed of 28 exons and can encode a protein of 1280 amino acids. Mutational analyses have revealed that the MDR1 gene is highly polymorphic and it is extensively used to investigate P-gp structure-function relationships. The first systematic SNP screening of the MDR1 gene revealed 15 different exonic and intronic SNPs in a healthy Caucasian population. Moreover, a total of 50 SNPs and 3 sertion/deletion polymorphisms have been reported in the MDR1 gene in recent years. Among them, it appears that researchers are more interested in only 28 SNPs at 27 positions. All 28 exons, including the core promoter region and exon-intron boundaries ranging from 49 to 587 bp, were sequenced using specific oligonucleotide primers derived from the original "wild-type" MDR1 sequence.Some of the polymorphisms in the MDR1 gene are 'silent', and cause no amino-acid changes (e.g. C1236T, C3396T and C3435T). However, some are found to change an amino acid (e.g. A61G, G1199A, A2956G, T3421A).

MDR1C3435T Genotype-Related P-gp Expression in cervical cancer

The different SNPs of the MDR1 gene, more mutation at position 3435 in exon 26 (C3435T), which is the only silent polymorphism identified so far that might influence P-gp expression in different human tissues and different races. Although the *MDR*1C3435T genotype has been associated with altered P-gp activity and has been extensively studied in different populations, the accurate molecular mechanism of the observed association is still poorly understood. Nevertheless, several mechanisms have been presumed. Firstly, the most popular one is that there exists a linkage between the C3435T SNP and other mutations elsewhere within the *MDR*1 gene, such as in the promoter/enhancer or intronic regions like T-129C, or in another exon like G2677T/A. Secondly, this silent mutation in *MDR*1C3435T may also reduce translation efficiency to influence functional consequences. Thirdly, it was presumed that the silent mutation might alter or regulate the processing and translation controlling of mRNA. Finally, it is possible that the C3435T transition can impact posttranscriptional modifications or is linked to an important sequence for mRNA processing.

Cervical Cancer

The cervical cancer is a disease in which the tumorogenisis form in the tissue of cervix. Cervix is another name for the neck of the womb. The womb and cervix are part of a woman's productive system. It is the lower part of the uterus and connects the body of the uterus to the vagina. The cervix is the opening to the womb from the vagina. It is really a strong muscle. Normally it is quite tightly shut, with only a small opening to let sperm in and flow from a period out of the womb. During labour, the cervix opens up to let the baby out. Different types of cervical cancers are Squamous cell carcinoma, Adenocarcinoma. Squamous cells are the flat skin-like cells that cover the outer surface of the cervix (the ectocervix). Adenomatous cells are gland cells that produce mucus. Symptoms of Cervical Cancer are, Vaginal bleeding in Between periods and after or during sex, Unusual vaginal discharge, Pelvic pain, Pain during sexual intercourse.

Stages of Cervical Cancer

Stage 0 (Carcinoma in Situ): In stage 0, abnormal cells are found in the innermost lining of the cervix. These abnormal cells may become cancer and spread into nearby normal tissue. Stage 0 is also called carcinoma in situ.

Stage I: In stage I, cancer has formed and is found in the cervix only. Stage I is divided into stages IA and IB, based on the amount of cancer that is found.

Stage IA: A very small amount of cancer that can only be seen with a microscope is found in the tissues of the cervix.In stage IA1, the cancer is not more than 3 millimeters deep and not more than 7 millimeters wide. In stage IA2, the cancer is more than 3 but not more than 5 millimeters deep, and not more than 7 millimeters wide.

Stage IB: In stage IB, cancer can only be seen with a microscope and is more than 5 millimeters deep or more than 7 millimeters wide, or can be seen without a microscope.

Stage II: In stage II, cancer has spread beyond the cervix but not to the pelvic wall Stage IIA: Cancer has spread beyond the cervix to the upper two thirds of the vagina but not to tissues around the uterus.

Stage IIB: Cancer has spread beyond the cervix to the upper two thirds of the vagina and to the tissues around the uterus.

Stage III: In stage III, cancer has spread to the lower third of the vagina, may have spread to the pelvic wall, and/or has caused the kidney to stop working. Stage IIIA: Cancer has spread to the lower third of the vagina but not to the pelvic wall.

Stage IIIB: Cancer has spread to the pelvic wall and/or the tumor has become large enough to block the ureters.

Stage IV: In stage IV, cancer has spread to the bladder, rectum, or other parts of the body.

Stage IVA: Cancer has spread to the bladder or rectal wall and may have spread to lymph nodes in the pelvis. Stage IVB: Cancer has spread beyond the pelvis and pelvic lymph nodes to other places in the body, such as the abdomen, liver, intestinal tract, or lungs.

Chemotheraphy

successful therapy outcome Chemotherapy is the most common mode of treatment provided to cervical cancer patients. Cisplatin Carboplatin ifosafamide, hycamtin, ifosamaide, paraplatin and platinol are commonly used in combination for chemotherapy in cervical cancer[13] and as mentioned earlier they are P-gp substrates. As studied in other cancers as leukemia[16], cervical[17] and lung cancers, MDR1 gene polymorphisms play an imperative role in in which the drugs used were P-gp substrate. SNPs in the

Table 1: The allele frequencies of MDR1 gene at exon 26 (3435) in controls and Cervical Cancer groups

Genotypes	Controls N=49	Cases N=6	Normal tissue n=5	Allele frequencies		
				Controls	Cases	Normal tissue
3435CC	45	3	3	C=0.95	C=0.56	C=0.83
3435CT	4	2	1			
				T=0.03	T=0.42	T=0.26
3435TT	0	1	0			

gene have shown a wide variety of differential expressions and functions as stated. Hence, we designed this investigation to determine the allele frequencies at the polymorphic site 1199 in the MDR1 gene in cervical tumors and in equal number of healthy blood controls from the Andhra region of South India; which may play a key role in predicting the therapy outcome with drugs, which are P-gp substrate.

Table 2: Chi-square test. P-values

Study group	Controls	Cases	Normal tissue
Controls	-	0.0001	0.2408
Cases	-	-	0.367

*p-value <0.05 is considered to be statistically significant

Table 3: fisher's-exact test. P-value

Study group	Controls	Cases	Normal tissue
Controls	-	0.002702	0.122959
Cases	-	-	0.567

*p-value <0.05 is considered to be statistically significant

MATERIALS AND METHODS

Selection of Patients

All were newly diagnosed cervical cancer cases taken during the study period and study cases were 24– 40 years age of women residents of Andhra Pradesh with no previous history of any cancer. The clinical pathologists confirmed the cervical cancer.

Biopsy Sample Collection

The samples were collected from patients who went for removal of tumor by surgery were collected from Basavatarakam cancer hospital and used for test samples.

Blood Sample Collection

5 ml of blood samples from healthy women were collected by venipuncture are used as controls.

Genomic DNA Isolation from Blood

Materials Required: Autoclaved eppendorff, Autoclaved micropipettes, Autoclaved micro tips, Autoclaved distill water, Eppendroff stand.

Reagents Preparation

RBC lysis buffer /TKM1 (100 ml) :Triss HCl (10mM)-0.121g, EDTA(2mM)-0.074g, KCl(10mM)-0.074g, MgCl2 (10mM)-0.2032g

Triss was first dissolved in few ml of autoclaved distilled water and the PH was adjusted to 7.6. by using 0.1% HCl. Then EDTA was dissolved followed by other chemicals

Cell lysis buffer/TKM2 (100ml): Triss HCl (10mM)-0.121g, EDTA (2mM)-0.074g, KCl (10mM)-0.074g, MgCl2 (10mM)-1.203g, NaCl(0.4M)-0.464

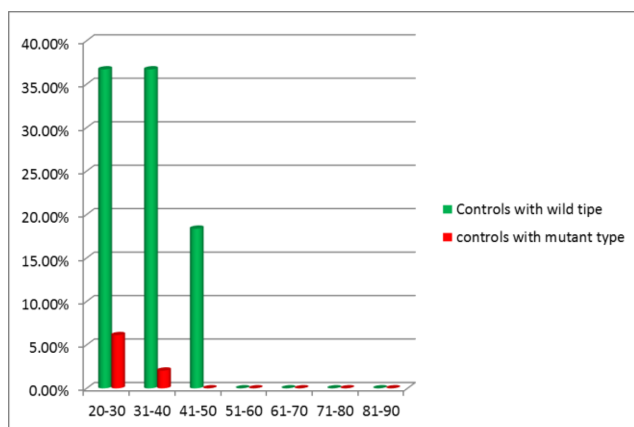
Triss was first dissolved in few ml of autoclaved distilled water and the PH was adjusted to 7.6. by using 0.1% HCl. Then EDTA was dissolved followed by other chemicals and the final volume was made up to 100ml with distilled water. 3.10% SDS (10ml): 1gm of SDS is dissolved in 10 ml of autoclaved distilled water. 4.6M of NaCl(25ml):8.756g of NaCl is dissolved in 25 ml of autoclaved distilled water. 5.TE Buffer (25ml): tris(10mM)-0.030g,EDTA(1mM)-0.009g. Tris was first dissolved in few ml of autoclaved , after adjusting the PH at 8,EDTA was dissolved, and the volume was made up to 25ml. 70% Ethanol – Dissolved 7ml of absolute Ethanol in 10 ml of distilled water.

Principle: RBC Lysis Buffer and Triton X 100 is used to remove the RBC's: Since RBC has no charge on their plasma membrane, non-ionic detergent called, triton X 100 removes them out. KCl and MgCl2 in TKM1 helps in lysis of the RBC cell membrane and EDTA acts as a divalent ion chelator (it contains di sodium atom). Hence it helps in deactivating the metallozymes as DNAses. Tris acts as a buffering agent maintaining the PH at 7.6 for the proper function of the lysis buffer. In addition,it helps in solubility of the ions so that they do not precipitate out.

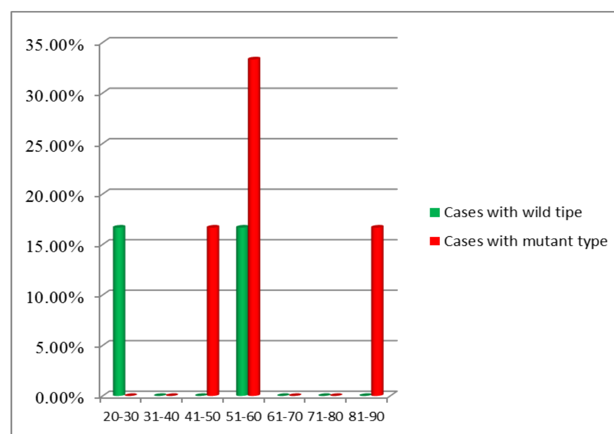
Centrifugation at 10000 rpm for 5 min, after incubation with RBC lysis buffer step separates out the lysed RBC's in the supernatant layer and intact lymphocytes precipitate out as pale colored pellet. TKM2 and 10% SDS are used to lyse the lymphocytes, TKM2 or Cell lysis buffer has a higher concentration of MgCl2, KCl & NaCl to lyse both the cell and the nuclear membrane. KCl also acts as a solubilizer of proteins. NaCl act as extractor of RNA and used in salting out of proteins. SDS acts as anionic detergent and both acts anionic lymphocytic cell membrane and help in their lysis deactivate the negatively charged proteins. 6 M NaCl is added to precipitate the proteins by salting out method. Centrifugation at 10000 rpm for 5 min helps in the precipitation of the aggregates of the proteins and cell debris as pellet and the supernatant contains the DNA strands in a solubilized form. Addition of the supernatant to cold absolute ethanol dehydrates the DNA and it is extracted as visible stands, The visible DNA threads are precipitated as pellet, washed using 70% alcohol, and again precipitated to remove agents like MgCl2, EDTA, KCl, NaCl that can inhibit Taq polymerase during the PCR of these samples.

Procedure

Take 300 µl of blood sample in eppendorff and Add 600 µl of TKM1 and one drop of 100% Triton X 100 to it, mix well, and incubate for 5 min. Centrifuge at 10000 rpm for



Graph1: Distribution of wild (CC) and mutant (CT or TT) genotypes at 3435 codon among controls over specified age range.



Graph 2: Distribution of wild (CC) and mutant (CT or TT) genotypes at 3435 codon of MDR1 gene among cervical cancer patients.

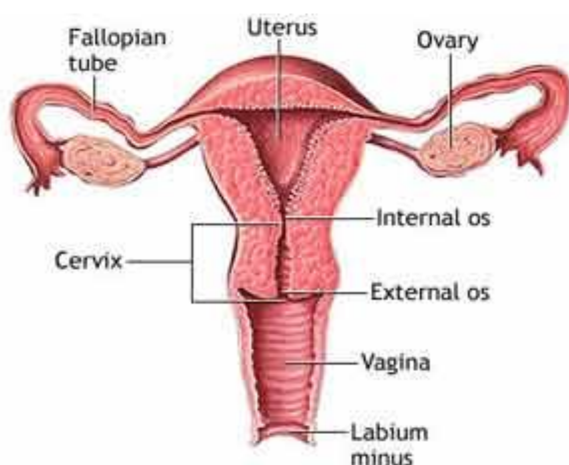
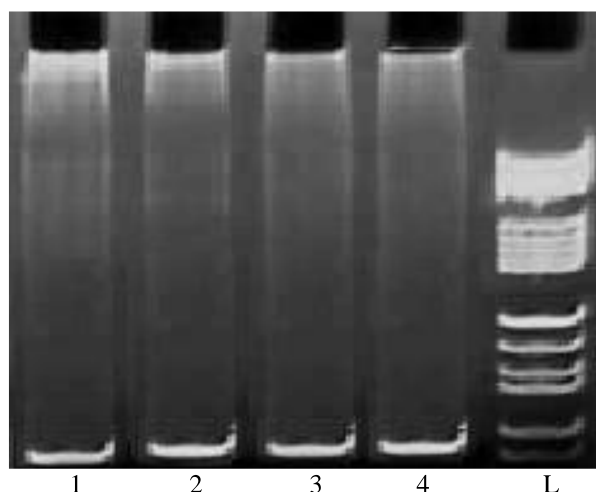


Fig.1: Female reproductive system



1 2 3 4 L

Fig.2:gel electrophoresis of PCR sample

5 min, and then discard the supernatant. To the pellet add 800µl of TKM1 add repeat the steps 2 and 3 until a white pellet is obtained. To the pale pellet, add 200 µl of TKM2 and 80µl of 10% SDS and incubate for 30 min. Add 80 µl of 6M NaCl and mix well by tapping for 5 min. Centrifuge at 10000 rpm for 5 min. Transfer the supernatant carefully to 680 µl of cold absolute ethanol. Centrifuge at 10000 rpm for 5 min. Discard the supernatant, add 300 µl of 70% absolute ethanol to DNA pellet. Centrifuge at 10000 rpm for 5 min and air dry the pellet. To the dry pellet add 50 µl of TE buffer for hydration of DNA and preserve at freezing temperature.

Genomic DNA Isolation from Tumer Tissue

Material required: All the material required in DNA isolation from blood cell are required along with Precleaned slides, Surgical blades, forceps.

Reagent Preparation

The reagents used are similar to that used in isolation of DNA from blood cells.

Principle: The principle of the procedure is similar as in case of isolation of DNA from blood cells

Procedure: Cut out 3mm of diameter of the given tissue on the precleaned slide. The tissue should be fat free or the yellow fatty tissue part should be completely trimmed out. The tissue piece is minced properly using the surgical blade to semi solid paste. Depending on the coloration or the bloodstain content of the tissue RBC lysis or directly cell lysis buffer treatment is done. Add 600 µl of TKM1 and one drop of 100% Triton X 100 to it, mix well, and incubate for 5 min. Centrifuge at 10000 rpm for 5 min, and then discard the supernatant. To the pellet add 800 µl of TKM1 add repeat the steps 2 and 3 until a white pellet is obtained. To the pale pellet, add 200 µl of TKM2 and 80 µl of 10% SDS and incubate for 30 min. Add 80 µl of 6M NaCl and mix well by tapping for 5 min. Centrifuge at 10000 rpm for 5 min. Transfer the supernatant carefully to 680 µl of cold absolute ethanol. Centrifuge at 10000 rpm for 5 min. Discard the supernatant, add 300 µl of 70% absolute ethanol to DNA pellet. Centrifuge at 10000 rpm for 5 min and air dry the pellet. To the dry pellet add 50 µl of TE buffer for hydration of DNA and preserve at freezing temperature.

Detection of DNA in The Isolated Samples Using 0.8% of Agarose Gel by Electrophoresis

Materials Required: Horizontal Electrophoresis Unit Gel plate, Combs, Adhesive tapes, 10 µl micropipette and autoclaved tips.

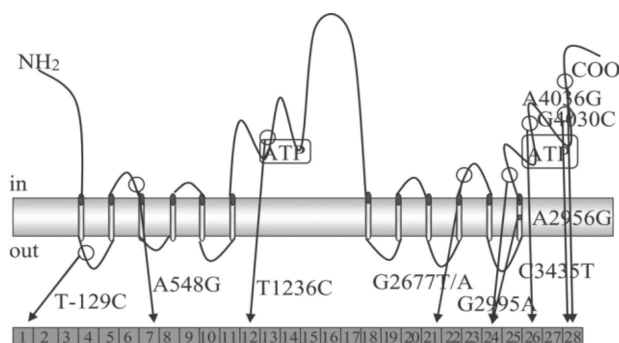


Fig. 3: Schematic representation of P-gp secondary structure and the positions of some important polymorphisms in the human *MDR*1 gene

Reagent preparation: 10x TAE Buffer (100) ml: Solution A: dissolve 19.36g of Tris buffer in 50ml of water. Solution B: dissolve 1.86g of EDTA in 10ml of water. Solution C: add 8ml of B to solution A and add 4.36ml of acetic acid. Then make up the volume to 100ml with water. 1XTBEbuffer: Dissolve 30ml of 10X TAE Buffer in 270ml of water to make 1:10 dilution. 0.8% Agarose: Dissolve 0.2g of Agarose in 25ml of 1XTAE Buffer. 1% Ethidium bromide solution: Dissolve 0.1g of ethidium bromidein 10 ml water.Gel loading solution and dye used is 6Xconcentrate is obtained readymade.

Principle: Electrophoresis: an electric field is developed across the Agarose gel with incorporated DNA samples. The DNA being negatively charged migrates towards anode. The Ethidium bromide acts as an interchelating agent and incorporates itself in to the DNAstrands. Since Ethidium bromide fluoresce under the sample. In addition it increases the density of the mixture, so that they reside down at the bottom of the well and are diffused in the gel.

Procedure: Close the open side s of the gel plate using adhesive taps. Place the combs. Add 10 µl of ethidium bromide solution to the cold molten Agarose and pour it in the gel plate. keeps it resting for casting of the gel for 15-20 minutes. Remove the taps and comb carefully. Pour the 1X TAE Buffer in the unit tank and place the gel placing the well at cathode end. Mix 1.5 µl of the loding concentrate with 4.5 µl of the DNA sample on a piece of parafilm. Add 5.0 µl of the mixture in to the well. Connect the wires and set the volts at 60. Run the gel at 60 V for 20-30 minutes. Observe the gel under UV in a transiliuminator.

Polymerase Chain Reaction Of The Specific Exon11 Using A Thermocycler

Polymerase chain reaction *invitro* was designed first by Karry Mullis in 1983. It follows the process of DNA replication using temperature variations with a help of a themocycler. This process include five major steps, at specific accurate temperature for each step for exact specificity the amplification or duplication of the specific DNA sequence or gene out of the whole genomic DNA sequence. This is possible by using specific complementary forward and reverse primers that specified the region of duplication. The enzyme used for the amplification is generally consists of 3'end to 5'end extention and 5'end to 3' end exonuclese activity. The enzyme used is called Taq polymerase, which is extracted from thermo stable bacteria Thermus aquaticus, generally found in hot springs. This makes the enzyme thermo stable and it can work actively at higher temperatures at which the double strand remains denatured after the denaturation step at 94˚C. Denaturation of double strands occurs by melting of the interstand hydrogen bonds in between the bases. The procedure includes denaturation, primer annealing, extension and renaturation for a single round of replication. These steps are orderly repeated to obtain number of replication required by variation of temperatures. The specific temperature of each step should not coincide with other. The initial denaturation generally occurs at 94 ˚C for about 5 minutes for denaturation of the whole genomic DNA.

MATERIALS

PCR mixture of 50 µl for each tube: Nuclease free water - 32.75 µl, Taq buffer (10X)-5 µl, MgCl2 -8 µl, dNTP's mix-1 µl, Forward primer - 1 µl, Reverse primer - 1 µl, Taq DNA polymerase – 0.25 µl, Template DNA - 1 µl.

PCR programme: Initial denaturation - 94˚C for 5 minutes, Denaturation -94˚C for 30 seconds , Annealing - 72˚C for 30 seconds, Extension - 72˚C for 45 seconds, Final Extension - 72˚C for 7 minutes.

Single strand confirmatory polymorphism (SSCP)

Principle: In denatured condition, each single strand of a double strand DNA molecule can undergo intrastrand hybridization by formation of hydrogen bonds with in the complementary bases. This leads to the formation of specific configuration other than the linear one. Change of even one nucleotide can effect the configuration of the strand. This change of configuration detecting change of polymorphs of nucleotides can be detected by the change in the movement of the denatured strands along an appropriate concentration of polyacrylamide gel in an electric field. This process is called as single strand confirmatory polymorphism (SSCP) type of electrophoresis.

Reagent Preparation: 50% Acryl amide solution- Dissolve 49g of Acryl amide and 1g of Bis-Acryl amide in water and make up the volume to 100ml.

10X TBE Buffer- Dissolve 1.86g of EDTA in 10ml of water adjust the pH to 8. Dissolve 10.8g of Tris and 5.4g of Boric acid in water and add 4ml EDTA solution. Then adjust the volume to 100ml. 10% Ammonium per sulfate- Dissolve 100mg Ammonium per sulfate in 1ml water.

1X TBE Buffer (400ml)- Dissolve 40ml 10X TBE Buffer in 360ml of water. Denaturing buffer/ loading dye- 25mg Xylene cyanol, 25mg Bromophenol blue are dissolved in 9.5ml of Formaldehyde.

Procedure

Denaturation of Samples: 5 µl of DNA solution (PCR product) is mixed with 15 µl of denaturing buffer and denatured at 94˚C for 5 minutes and snap cooled on ice. Casting the gel: Preparation of 12% non-denaturing polyacrylamide gel (49:1) {10ml}- Dissolve 2.69ml acryl amide solution, 0.976ml 10X TBE,114 µl 10% APS, 11 µl TEMED in 6.209ml water.

Mix the reagents as specified above and pour in to the vertical gel mould avoiding air bubbles. Insert the combs. Leave the mould undisturbed for an hour for polymerization. Remove the combs and fix the gel plate to the unit. Fill 1X TBE buffer in the upper and lower tank and prerun at 175 volts for hour.

Electrophoresis: Load 20 µl of the denatured sample along with an undenatured control in to the wells.Carry out the electrophoresis at 175 volts.45-50amps for 4-5 hours.

Silver Staining:

Submerge the gel in to 40% methanol for 20 minutes in a tray. Pour off the methanol and add 160mM HNO3 (1ml in 100ml water)solution for 5 minutes. Rinse the gel with distilled water for 5 minutes. Decant the water and stain the gel by immersing in 0.2% AgNO3 solution in dark and constant shaking for 10 minutes. Decant the stain and rinse the gel in distille water for 5 minutes. Add 50ml of prechilled developer solution (2.9g Sodium carbonate and 50 µl formaldehyde in 100 ml water) with constant shaking till the desired bands are obtained. Add stop solution to cease the staining reaction(5ml acetic acid,25ml methanol, 20ml water).

RESULTS

Case Report Form For Cervical Cancer

IP. No.:, Date:

1. Name:
2. Age/Sex:
3. Occupation:
4. Pan masala:
5. Smoker/Non-smoker :
6. Alcoholic/Non-alcoholic:
7. HPV infection statues : HPV +ve, HPV –ve
8. Stage:
9. Tumor size(cm): <5, >5
10. Histology: squamous, adenocarcinoma, adenosquamous
11. Differentiation: Well, Moderate, Poor
12. Hemoglobin at diagnosis (g/dl): <12.6 - >12.6
13. Pelvic nodes: positive - Equivocal - negative

DISCUSSION

The human multi drug resistant gene (mdr1) encodes a 170-k da integral membrane protein called p-glycoprotein that mediates ATP dependent substrate efflux18. A wide variety of natural compounds and lipophilic xenobiotics and drugs are substrates of p-gp recently several polymorphisms of mdr1 gene have been identified and found to result in increased resistance in certain cancers . The most common polymorphisms include exon 21 2677(G/T/A) exon12 1236(C/T) exon 1- 129(T/C) exon11-1199 (G/A) and exon 26- 3435(C/T) 19. In the present study we determined the prevalence of the C-T transition at 3435 codon in exon 26 of mdr1 gene in cervical cancer cases. We performed the mutational analysis on three separate groups as 49 healthy female controls, 6 cervical cancer patients and 5 normal surrounding tissues. The specific PCR primers used to amplify the whole exon 26. Hence we found significant higher prevalence of G3435T polymorphism in cancer cases compared to controls. Only one of the surrounding normal cervix tissue has shown a mutant genotype. In comparison only 25 % of the healthy controls have shown the mutant genotype. To evaluate the statistical significance of the allelic distribution of the SNP, under study among the cases, controls, healthy tissues we performed chi-square and fisher's exact test. The results of these statistical analysis revealed that there is a significant genetic heterogensity among controls and tumor genotypes as well as in surrounding normal tissues and tumor genotypes. The significant genetic hetrogencity among the surrounding normal tissues and tumor indicates the emergence of genomic instability during tumorogensis. Where as both the tests have shown no significant difference between the genotypic data of healthy controls and normal surrounding tissues, as per given table 2 and 3 Furthermore we stratified our polymorphism data on the basis of demographic details of the cases and controls to determine the pattern of occurrence of the SNP according to age. We grouped the wild type and mutant cases and controls on the basis of age ranges as shown in graph 1and 2. We observed that highest percentage of mutant mdr1 genotype at 3435 codon in controls belonging to the age of 30-40 years where as wild type genotype are comparitatively more in the age range of 21-30 years. We also observed that the about 50% of the cases have shown the SNP and of the age of 40-50 years range.

CONCLUSION

In humans, a nucleotide at position 3435 on exon 26 of MDR1 is reportedly polymorphic, since it can substitute cytosine base (C) with thymine base (T), yielding 3 genotypes: CC genotype (wild type), CT genotype (heterozygous type) and TT genotype (mutant type). Individuals with the TT genotype have been found to be at increased risk of several cancers. The multidrug resistance 1 gene (MDR1) plays an important role in regulating the expression and function of P-glycoprotein (P-gp), an efflux-pump on the cell membrane, to excrete toxins and drugs from cells. Therefore, the purpose of this study was to investigate the association between MDR1 (C3435T) polymorphism and the risk of CVC among females in Andhrapradesh. The age-matched study subjects were divided into two groups--normal controls and CVC patients. Genomic DNA, which had been extracted from the peripheral blood leukocytes of each subject, was analyzed for MDR1 (C3435T) polymorphism by PCR technique. It was found that individuals with the TT genotype were at higher risk of CVC, than those with the CC genotype. Present study showing results high prevalence of the C-T transition at 3435 codon in exon 26 of mdr1 gene in cervical cancer cases. We observed mutational analysis on three different groups of healthy female controls, cases, and surrounding normal tissues. Hence we conclude that the allele frequencies (C or T) at the polymorphic sites had shown a significant prevalence of mutant A allele (0.5) in the patient groups when compared to healthy controls (0.9) and surrounding normal tissues of the same patients (0.8). From these results we conclude that CT genotype has significant correlation with

cancer development of mutant mdr1 genotype at 3435 codon at the age of 40-50 years range.

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