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Research Article

POLYMORPHISM OF MDR1 GENE IN CERVICAL CANCER CASES AND CONTROLS

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ABSTRACT

The MDR1 multi drug resistant gene, transporter represents one of the better-characterized drug transporters that play an important role in protecting the body against xenobiotic substances and toxic compounds. P-glycoprotein (P-gp) is the Trans membrane multi drug transporter encoded by the MDR1 gene. Generally, it is expressed in normal tissues with excretory function as Intestinal, Renal and Colorectal and plays a major role in Steroid metabolism. MDR1 gene located at Chromosome 7q21.12 and of 209 kb. The gene transcribes into a 4.63 kb mRNA which codes for a 1280 amino acid chain. This MDR1 gene has 28 amino acid coding exons and 1 non-coding exon. In the present study we determined the prevalence of the G-A transition at 1199 codon in exon 11 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. There are 25 different polymorphisms were reported in MDR1 gene, 12 of the polymorphisms do not alter the protein sequence; seven polymorphisms are located in introns 4, 6, 12, 16, & 17 close to exon boundaries (Fig: 1). Four polymorphisms detected in exon 2,5,11 and 21 are responsible for protein alteration. The G2677C/T polymorphism in Exon 21 has been found to alter the protein chain at Ala893Ser/Thr and relate to increase incidence of inflammatory bowel diseases and cervical Cancer. Exon 2 contains a polymorph that changes Asn 21 to Asp, which alters the net charge on the protein, Exon 5, showed heterozygosis with a polymorphism that changes Phe 103 to Leu, which influence the substrate recognition and exon 11 have a polymorphism (G1199A) at Ser 400 to Asn alters the net charge and substrate specificity.

Keywords: P-Glycoprotein, polymorphisms, exon11, cervical cancer patients.

1. INTRODUCTION

1.1 MDR1 GENE AND IT' S PRODUCT

The MDR1 multi drug transporter represents one of the better-characterized drug transporters that play an important role in protecting the body against xenobiotic substances and toxic compounds. P-glycoprotein (P-gp) is the Trans membrane multi drug transporter encoded by the MDR1 gene. Generally, it is expressed in normal tissues with excretory function as Intestinal, Renal and Colorectal[1] and plays a major role in Steroid metabolism[2]. MDR1 gene located at Chromosome 7q21.12 and of 209 kb. The gene transcribes into a 4.63 kb mRNA which codes for a 1280 amino acid chain. This MDR1 gene has 28 amino acid coding exons and 1 non-coding exon[3].

P-gp was originally discovered in drug resistance Chinese-Hamster cervical Cell mutants[4].This gene expression has also been observed in the malignant cells, which show de *novo* or acquired resistance to drugs. Over expression of P-gp in cancer cells causes a decrease in drug accumulation, thereby mediating cellular resistance to a variety of anticancer agents[5].

The overall MDR1 activity controlling P-gp dependent drug transport depends on two parameters: the level of expression of MDR1 gene controls the amount of protein that is synthesized in the cells and the functionality of the MDR1 encoded P-gp, which determines the substrate recognition and their effective transport[6]. Transient exposure to drugs transcriptionally increases the level of expression of MDR1 gene. P-gp restricts the transport of drugs by an energy dependant pathway and it acts as a normal physiologic canalicular membrane transport[7]. The substrate list of P-gp includes many of the commonly used anti neoplastic drugs as Doxorubicin, taxols, Cisplatin etc.[8]. Hence, expression and functionality of MDR1 gene has a particular importance in cancer therapy.

Mutational analysis of MDR1 gene revealed polymorphic and was extensively used to investigate P-gp structure – function relationships. The sensitivity of the tumor cell towards chemotherapy often correlates inversely with increased MDR1 expression. Allelic differences and sequence alterations could influence expression levels especially promoter or enhancer, sequences that responsible for processing of pre-mRNA and its stability. Within this gene has been variously associated with differences in MDR1 expression, function, and drug response as well as disease susceptibility.

There are 25 different polymorphisms were reported in MDR1 gene, 12 of the polymorphisms do not alter the protein sequence; seven polymorphisms are located in introns 4, 6, 12, 16, & 17 close to exon boundaries (Fig: 1). Four polymorphisms detected in exon 2,5,11 and 21 are responsible for protein alteration . The G2677C/T polymorphism in Exon 21 has been found to alter the protein chain at Ala893Ser/Thr and relate to increase incidence of inflammatory bowel diseases and cervical Cancer. Exon 2 contains a polymorph that changes Asn 21 to Asp, which alters the net charge on the protein, Exon 5, showed heterozygosity with a polymorphism that changes Phe 103 to Leu, which influence the substrate recognition and exon 11 have a polymorphism (G1199A) at Ser 400 to Asn alters the net charge and substrate specificity[10]. MDR1 gene over expression has been reported in cervical cancer and also found to be associated with the disease progression[11].

Mdr1 gene

The MDR1 multi drug transporter is one of the better characterized of the ATP-binding cassette (ABC) family of transporters. The MDR1 transporter has been found to efflux a wide variety of substrates including anti- neoplatic agents anti-arrhythmics, anti-depressants, anti-psychotics and anti-virals.[20, 21]

The MDR1 gene spans 200 kb and is located at chromosome 7p21. It comprises 28 exons and possesses two transcription start sites and two promoters that are 100 kb apart. In the past few years, great interest has been generated in the identification and characterization of single nucleotide polymorphisms (SNPs) in the MDR1 gene and its association with drug response, clinical outcome and susceptibility to certain diseases. These studies examined SNPs from the downstream promoter to the 3' end of the gene and primarily concentrated on SNP e26/3435(C/T), as well as SNPs e21/2677(G/T/A), e12/1236(C/T) and e1/–129(T/C). Except for SNP e1/–129(T/C), low

frequency (<10%) exotic SNPs have been reported in only one or two ethnic groups and may represent ethnic-specific or geographically restricted SNPs. Of the high-frequency exonic SNPs, only three SNPs, e12/1236(C/T), e21/2677(G/T/A) and e26/3435(C/T), have been reported to occur in different ethnic populations and at different frequencies.[21]

The high but ethnically variable frequency SNP e26/3435(C/T) has been variously associated, either alone or in combination with SNPs e21/2677(G/T/A), e12/1236(C/T) or e1/–129(T/C), with differences in MDR1 expression and functiondrug response and clinical outcome, and with susceptibility to various diseases. SNP e26/3435(C/T), however, does not result in an amino acid change and conflicting results of positive, negative and no associations have been reported. We previously demonstrated strong but varied linkage disequilibrium (LD) of SNP e26/3435(C/T) with SNPs e21/2677(G/T/A) and e12/1236(C/T) in three different Asian populations, and proposed that SNP e26/3435(C/T) may not be the causal modulator of previously reported functional differences but may instead be in strong linkage disequilibrium with different unidentified causal SNPs in the different study populations.[21]

Polymorphism at G1199A

The significance of the human multi drug resistance gene (MDR1) G1199A polymorphism, resulting in a Ser400Asn modification in P-glycoprotein (P-gp) [22] Human multi drug resistance gene (MDR1) G1199A polymorphism (amino acid change Ser400Asn) alters P-glycoprotein (P-gp)-dependent Tran epithelial permeability and uptake kinetics of HIV protease inhibitors (PI), it was found by using recombinant epithelial cells expressing wild-type MDR1 ($MDR1_{wt}$) or the G1199A variant ($MDR1_{1199A}$). Methods using a recombinant expression system developed previously, the trans epithelial permeability and uptake kinetic parameters of five PI, amprenavir, indinavir, lopinavir, ritonavir, and saquinavir were estimated across polarized epithelial cells. Results For all PI, the trans epithelial permeability ratio (basolateral-to-apical transport divided by apical-to-basolateral transport) was significantly greater in $MDR1_{1199A}$ cells than $MDR1_{wt}$ cells: amprenavir (1.7-fold), indinavir (1.8-fold), lopinavir (1.5-fold), ritonavir (2.8-fold), and saquinavir (2.1-fold). However, the impact of G1199A on P-gp activity appeared to primarily influence drug permeability in the apical-to-basolateral direction. Kinetic analysis of ritonavir and saquinavir uptake by $MDR1_{wt}$- and $MDR1_{1199A}$-expressing cells showed that V_{max} was similar, while uptake K_m was significantly higher in cells expressing the G1199A variant suggesting that alterations in P-gp-dependent efflux mediated by G1199A were due to changes in transporter affinity.[23]It is also proved in case of paclitaxel and carboplatin the allelic distribution of the G1199T/A and other polymorphisms in exons 11 and 12 of the ABCB1 gene in cervical cancer patients treated with paclitaxel and carboplatin alters the permeability of the drug. The SNPs C1236T, G1199T/A, and A1308G were determined using Pyrosequencing in 51 patients with advanced cervical cancer and correlated to the progression free survival. The G1199T/A SNP was found to affect the progression free survival[24]

1.2 CERVICALCANCER

Cervical cancer develops in the lining of the cervix, the lower part of the uterus (womb) that enters the vagina (birth canal). This condition usually develops over time. Normal cervical cells may gradually undergo changes to become precancerous and then cancerous. Cervical intraepithelial neoplasia (CIN) is the term used to describe these abnormal changes. CIN is classified according to the degree of cell abnormality. Low-grade CIN indicates a minimal change in the cells and high-grade CIN indicates a greater degree of abnormality. CIN may progress to squamous intraepithelial lesion (SIL; condition that precedes cervical cancer) or to carcinoma in situ (cancer that does not extend beyond the epithelial membrane). SIL is also classified as low-grade or high-grade. High-grade SIL and carcinoma in situ may progress to invasive carcinoma (cancer that has spread to healthy tissue).[12, 13]

The incidence of invasive cervical cancer in the United States, where approximately 13,000 cases of invasive cervical cancer and 50,000 cases of cervical carcinoma in situ (i.e., localized cancer) are diagnosed yearly.[14]

The incidence of cervical cancer in India 17.1 women per 100,000 populations with cervical cancer in India 1993-97 (Surveillance and Risk Assessment Division, CCDP, Health Canada) [15]

1.3 **CHEMOTHERAPHY**

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 a imperative role in successful therapy outcome in which the drugs used were P-gp substrate. SNPs in the 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.

3.0 MATERIALS AND METHODS

3.1 Study population and sample collection

Cervical cancer patients were assessed on the basis of clinical examinations as well as pathological examinations. The cervical Cancer Study is a case-control study conducted in South India. A total of 25 cervical cancer patients and 25 age-matched controls were enrolled in the study.

3.2 Inclusion and exclusion criteria

All cervical cancer cases were newly diagnosed during the study period, and meeting the following criteria was eligible for this study: 22–40 years age of women residents of Andhra Pradesh (south India), with no previous history of any cancer. The cervical cancer patients studied here had not been exposed to chemo- and/or radiotherapy before. They underwent clinical examinations at the Various Hospitals in Hyderabad. Written informed consent was obtained from all subjects, and relevant ethical committees approved the study benefit of humans in general. Two senior pathologists confirmed all diagnoses.

3.3 Collection of biopsy samples

The biopsy samples from patients who underwent surgery for removal of tumor (from cervix) were collected from various institutes like Indo American Cancer Institute & Govt. BIBI cancer Hospital and was used as test samples. The cancer samples collected were all diagnosed with HPV infection

3.4 Collection of blood samples

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

4.0 GENOMIC DNA ISOLATION FROM BLOOD

4.2 Reagents Preparation

4.2.1 RBC lysis buffer /TKM1 (100 ml)

Triss Hcl (0.121g) 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 (2mM)-0.074g was added to already prepared mixture of KCl (10mM)-0.074g and $MgCl_2$ (10mM)-0.2032g

4.2.2 Cell lyses buffer/TKM2 (100ml)

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 as above and the final volume was made up to 100ml with distilled water.

4.2.3 10% SDS (10ml): 1gm of SDS is dissolved in 10 ml of autoclaved distilled water.

4.2.4 6M of NaCl (25ml): 8.756g of NaCl is dissolved in 25 ml of autoclaved distilled water.

4.2.5 TE Buffer (25ml): 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.

4.2.6 70% Ethanol: Dissolved 7ml of absolute Ethanol in 10 ml of distilled water.

4.2.7 Procedure

Take 300 µl of blood sample in eppendorff. 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 and then 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.

4.2.8 GENOMIC DNA ISOLATION FROM TUMOR TISSUE

4.2.8.1 Material required:

All the material required in DNA isolation from blood cell is required along with the following.
Pre cleaned slides, surgical blades, Forceps

4.2.8.2 Reagent preparation

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

4.2.8.3 Procedure

Cut out 3mm of diameter of the given tissue on the pre cleaned 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.

5.0 DETECTION OF DNA IN THE ISOLATED SAMPLES USING 0.8% OF AGAROSE GEL BY ELECTROPHORESIS

5.1 Materials required

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

5.2 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 bromide in 10 ml water. Gel loading solution and dye used is 6Xconcentrate is obtained readymade.

5.3 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 loading concentrate with 4.5 µl of the DNA sample on a piece of Para film. 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 Tran's illuminator.

6.0 POLYMERASE CHAIN REACTION OF THE SPECIFIC EXON 11 USING A THERMOCYCLER

Polymerase chin 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. 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.

6.1 MATERIALS

Nuclease free water, Taq buffer (10X), $MgCl_2$, DNTP's mix, Forward primer, Reverse primer**.** PCR mixture of 50 µl for each tube.

Primer Sequence

Forward 5^1 GATTAATCATTTATCACTGTAC 3^1
Reverse 5^1 CTTAAAATTTGATTCTGTTTAG3^1

7. SINGLE STRAND CONFIRMATORY POLYMORPHISM (SSCP)

7.1 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) and **Denaturing buffer/ loading dye (**25mg Xylene cyanol, 25mg Bromophenol blues are dissolved in 9.5ml of Formamide and 0.5ml of dist' H_2O).

7.2 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 pre run at 175 volts for hour.

Electrophoresis

Load 20 µl of the denatured sample along with an un denatured 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 HNO_3 (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% $AgNO_3$ solution in dark and constant shaking for 10 minutes. Decant the stain and rinse the gel in distilled 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 stock solution to cease the staining reaction (5ml acetic acid, 25ml methanol, 20ml water).

RESULTS AND DISSCUSION

The human multi drug resistant gene (mdr1) encodes a 170-k da integral membrane protein called p-glycoprotein that mediates ATP-dependent substrate efflux[18]. 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 [18]. 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 G-A transition at 1199 codon in exon 11 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 exon11 produced a product of 111 base pairs

Lane1 .2.3.4 are having PCR samples corresponding to 111 base pairs lane 5 is ladder 100bp as per given table 6.

These amplified products were subjected to SSCP analysis in comparison to sequenced samples with the SNP

Figure 7: Lane1 is 1199GG lane2 is 1199GA lane3 is 1199AA lane 4 and 5 are referenced sequenced sample lane 6 is 100 bp ladder In previous studies it was reported that G1199A polymorphism of mdr1 gene Resulted in altered efflux for specific cyto toxic and HIV protease inhibitor drugs in normal and recombinant epithelial cell lines [25, 26]. As mentioned earlier p-gp expression has been detected in cervical cancer tissues in correlation to chemotherapy response and disease out come. Hence we hypothesized that the G1199A SNP which have been found to alter p-gp function may reveal specific pattern of efflux and response to specific anti neoplastic drugs in cervical cancer patients our results of determining the allele frequencies (G or A) at the polymorphic sites had been shown a significant prevalence of mutant A allele (0.5) in the patient groups when compared to healthy controls (0.13) and surrounding normal tissues of the same patients (0.10) we also observed that the alleles strictly fallowed hardy- Wein berg's rule in each study groups as per given table 3.

Hence we found significant higher prevalence of G1199A polymorphism in cancer cases compared to controls. Interestingly we found that all the tumor tissues harbored GA genotype, where as only one of the surrounding normal cervix tissue had shown the mutant genotype. In comparison only 26.5 % of the healthy controls have shown the mutant genotype. We also observed that the homozygous mutant (AA) genotype was not present in the study groups (cases and controls) drawn from southern Indian population. To evaluate the statitistical 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 4 and 5

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 7 age ranges as shown in graph 1and 2.

We observed that highest percentage of mutant mdr1 genotype at 1199 codon in controls belonging to the age of 31-40 years where as wild type genotype are comparitatively more in the age range of 20-30 years. We also observed that the about 50% of the cases have shown the SNP and of the age of 41-50 years range.

CONCLUSION

From the results of the present study we conclude that there is high prevalence of the G-A transition at 1199 codon in exon 11 of mdr1 gene in cervical cancer cases. From the results of the mutational analysis on three different groups namely healthy female controls, cases, and surrounding normal tissues we conclude that the allele frequencies (G or A) at the polymorphic sites had shown a significant prevalence of mutant A allele (0.5) in the patient groups when compared to healthy controls (0.13) and surrounding normal tissues of the same patients (0.10). From the results we conclude that GA genotype has significant correlation with cancer development and the highest percentage of mutant mdr1 genotype at 1199 codon at the age of 41-50 years range and these polymorphisms during the lifetime of the individuals, and the event of tumorogensis leads to further accumulation of this polymorphism.

Table 1: Composition Of pcr mixture (polymerase chain reaction)

S. No.	Ingredients	Quantity (µl)
1.	Nuclease free water	32.75
2.	Taq buffer (10X)	5
3.	$MgCl_2$	8
4.	DNTP's mix	1
5.	Forward primer	1
6.	Reverse primer	1
7.	Taq DNA polymerase	0.25
8.	Template DNA	1

Table 2: PCR program

S. No.	Stages	Temperature (°C)	Duration Time
1	Initial denaturation	94	5 minutes
2	Denaturation	94	15 seconds
3	Annealing	49	30 seconds
4	Extension	72	30 seconds
5	Final Extension	72	10 minutes.
6	Block	40	5 min

Table 3: The allele frequencies of MDR1 gene at exon 11 (G1199A) in controls and Cervical Cancer groups

Geno type	Controls N=49	Cases N=6	Surrounding Normal tissue N=5	Allele frequencies		
				Controls	Cases	Normal tissues
1199GA	13	6	1	G= 0.87	G=0.5	G=0.9
1199AA	0	0	0			
1199GG	36	0	4	A=0.13	A=0.5	A=0.1

Table 4: p- values obtained from Chi-Square test comparing each of the two groups

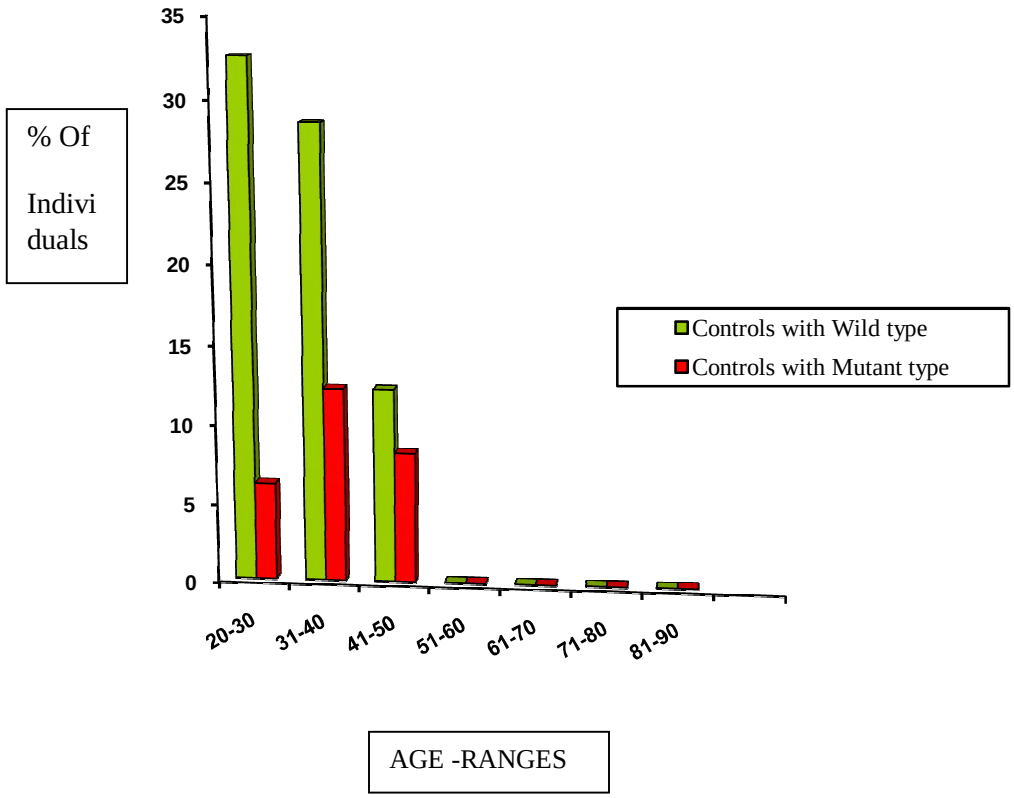
Study Group	Cases	Normal tissue
Controls	0.0018	0.8273
Cases	____	0.0343

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

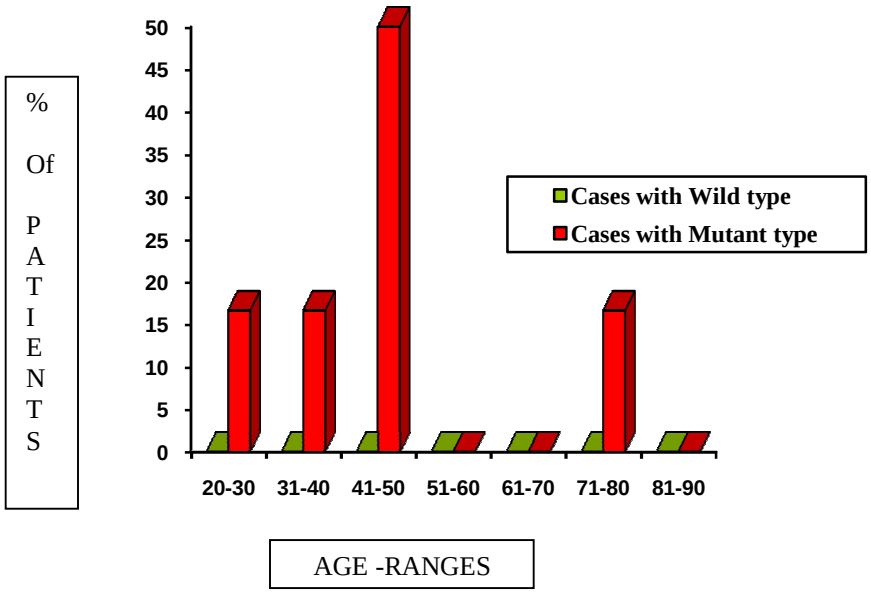
Table 5: p- values obtained from Fisher's – exact test comparing each of the two groups

Study Group	Cases	Normal tissue
Controls	0.000936	1.0
Cases	____	0.015

*p value <0.05 is considered to be statistically significant



Graph 1: Percentages of individuals with wild and mutant genotype at 1199 codon of MDR1 gene in the control group



Graph 2: Percentages of Cervical Cancer patients with wild and mutant genotype at 1199 codon of MDR1 gene

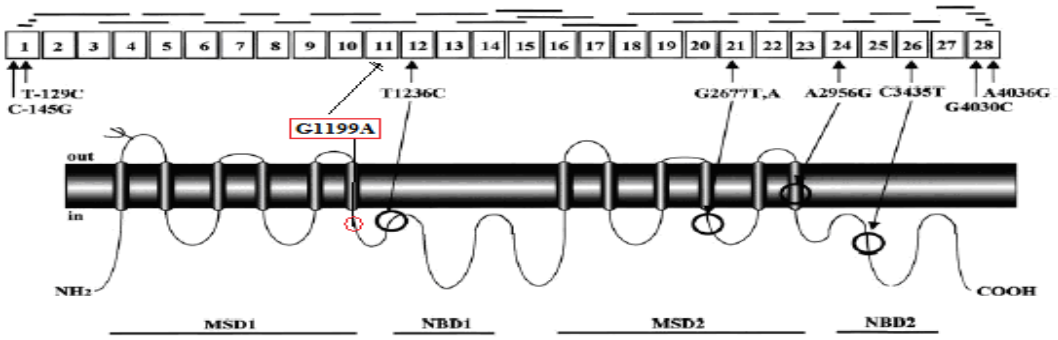


Fig. 1: Common SNPs in MDR1 gene and its location on p-glycoprotein

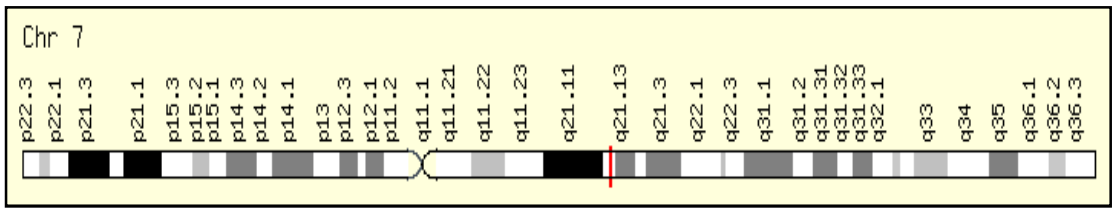


Fig. 2: Chromosomal location of MDR1 gene

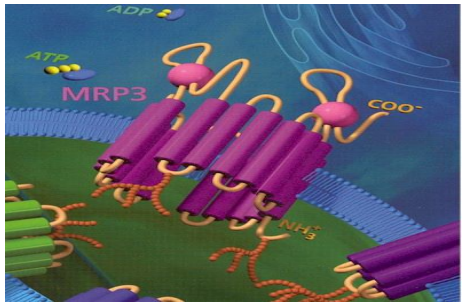


Fig. 3: Structure of MDR l genes

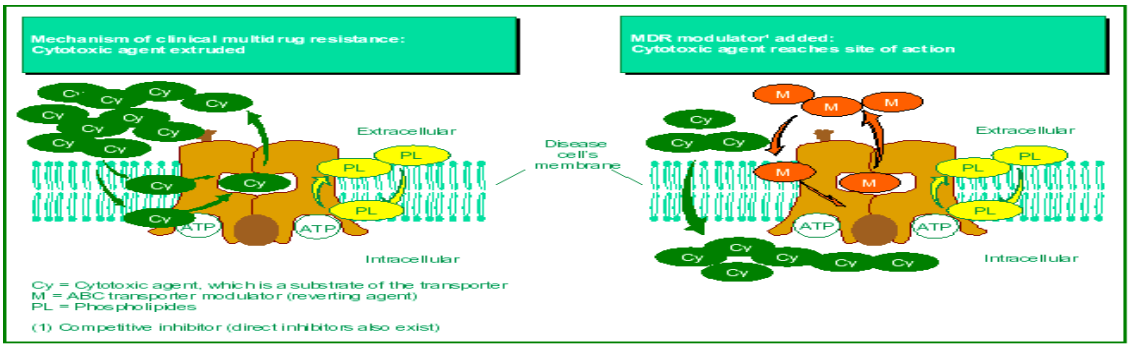


Fig. 4: Mechanism of clinical multi drug resistant: cytotoxic agent extruded

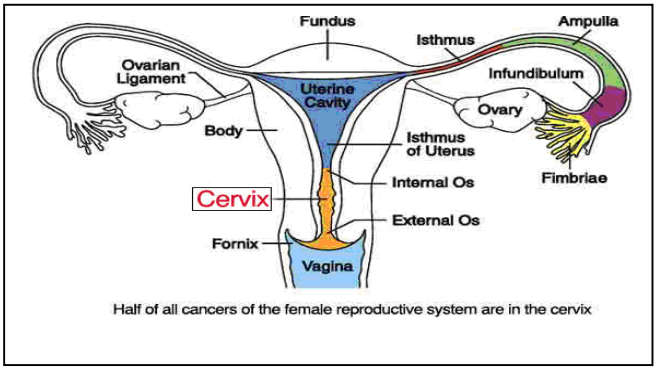


Fig. 5: Female Reproductive systems

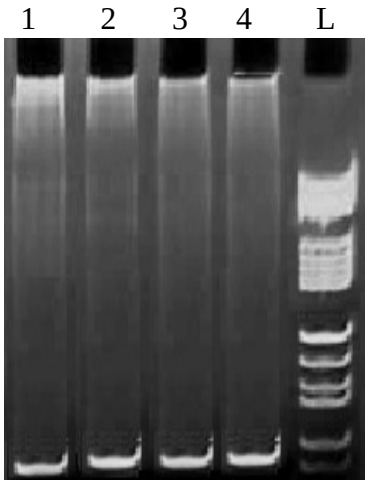
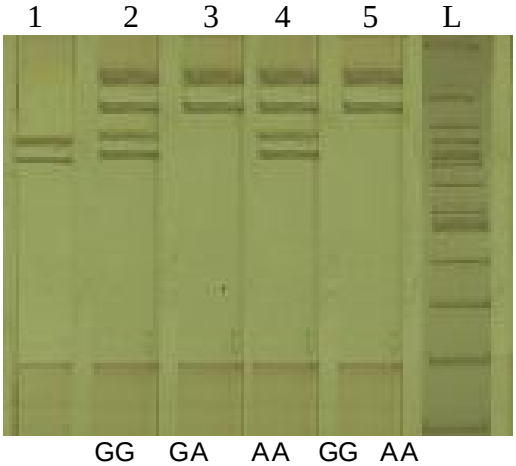


Fig. 6: 2% agarose gel electrophoresis of PCR sample



Lane1 .2.3.4 are having PCR samples corresponding to 111 base pairs lane 5 is ladder 100bp.

Fig. 7: 15% page gel electrophoresis of PCR samples

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