Investigation of BRCA1, BRCA2 Gene Mutations and P53 Gene Polymorphism in High-Voltage Transmission Line Workers

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Abstract

The aim of the present study is to investigate mutations in tumor suppressor BRCA1 and BRCA2 genes and P53 gene polymorphism in high-voltage power station workers and to determine the distribution of this polymorphism among these workers.

This study carried out on 35 high-voltage transmission line (HVTL) workers with a mean age of 36.6 ± 6.8 years. The data obtained from the exposure group were compared to the control group. Control group was created from volunteers who have same age range (35.4 ± 6.4 years) and socio-cultural level except occupational extremely low frequency magnetic field (ELFMF) exposure. DNAs were isolated from the blood samples collected from both groups and BRCA1 and BRCA2 gene mutations and p53 gene polymorphism were investigated. Isolated DNAs were used to amplify the mutation regions of exon 2 and exon 20 regions for BRCA1 and exon 2 for BRCA2 with polymerase chain reaction (PCR) and the single-strand conformational polymorphism (SSCP) method was used to detect the mutations. PCR and Restriction Fragment Length Polymorphism (RFLP) were used to determine P53 codon 72 Arg/Pro gene polymorphism. The exon 2 mutation of BRCA1 was detected in only 1 worker in the occupational exposure group. It should be noted that the worker was also cancer and receiving treatment. It remains unclear whether the mutation was cancer-related or a result of occupational exposure. However, the investigation on P53 gene polymorphism did not indicate any difference between the groups ($p > 0.05$).

In conclusion, BRCA1 and BRCA2 mutations were not observed among the high-voltage transmission line workers except one in this study. However, P53 gene polymorphism also showed no difference between groups. Finally, further studies are required to clarify the underlying causes of the mutation detected in a worker in this study group.

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Introduction

The transfer of electric power through long distance (from the main stations to living areas) is only possible by the high-voltage (HV) transmission lines. Transmission of HV to the distribution system is conducted through electric power distribution centers. Various medical conditions may be associated with certain factors such as extremely low-frequency magnetic fields (ELFMFs) occurring around the HVTL. This issue has been a matter of debate since the 1970s. Although it appears rather as a background issue against the introduction of mobile phones or base stations in daily life, it still remains a subject of increasing interest for scientists[1-4].

While the concerns regarding a possible association between childhood cancers and ELFMFs caused by HVTL is one of the subjects of intense scientific research, the risks of occupational exposure to such fields appears to be another matter of debate. Additionally, the mutations/polymorphisms in tumor suppressor genes which play a regulatory role in cell proliferation and differentiation are suggested to increase the risk of several cancer types[5-7]. The present study has been performed to investigate

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whether occupational exposure to ELFMFs causes mutations in tumor suppressor genes. Therefore the aim of this paper is to investigate whether occupational exposure to ELFMFs lead to gene mutations among technicians working at electric power distribution centers.

Materials and methods

The present study has been carried out on 35 volunteers with 36.6±6.8 years of age working at high-voltage transmission lines. However, a control group (35.4±6.4 years) was established in the same social status except ELFMFs exposure. Official and ethical permission was before obtained from the institution where the study subjects worked. However, the study group was accepted as occupational exposure group. Blood samples were collected from the all the subjects in the study, and DNAs were isolated from these samples to investigate BRCA1 and BRCA2 gene mutations. Isolated DNAs were used to amplify the mutation regions of exon 2 and exon 20 regions for BRCA1 and exon 2 for BRCA2 with polymerase chain reaction (PCR) and the single-strand conformational polymorphism (SSCP) method was used to detect the mutations. On the other hand, PCR and Restriction Fragment Length Polymorphism (RFLP) were used to determine the P53 codon 72 Arg/Pro gene polymorphism.

DNA Isolation

A total of 2 mL peripheral blood was collected from each study participant into ethylene diamine tetraacetic acid-containing tubes. DNAs were isolated by means of a DNA isolation kit (High Pure PCR Template Preparation Kit, Roche USA). DNA purity and quantity were determined as per the spectrophotometric absorbance values at 260 nm and 280 nm. DNA quality was investigated under UV light following EtBr staining and 0.8% agarose gel electrophoresis.

Exon 2 and exon 20 mutations of BRCA1 and BRCA2:

Polymerase chain reaction (PCR) was performed using appropriate primers for the regions containing the exon 2 and exon 20 mutation spots for BRCA 1

For exon 2; F: 5'-GAAGTTGTCATTTTATAAACCTTT-3' and R: 5'-TGTCTTTTCTTCCCTAGTATGT-3'
For exon 20; F: 5'-ATATGACGTGTCTGCTCCAC-3' R: 5'-GGGAATCCAAATTACACAGC-3')[5].

The BRCA2 region containing the exon 2 mutation (311 bp) was amplified using appropriate primers;
F: 5'-CCAGGAGATGGGACTGAATTAG-3' and
R: 5'-CTGTGACGTACTGGGTTTTTAGC-3')[6].

PCR was performed with 0.5 nmol from each primer, 0.2 mM dNTP, 1.5 mM MgCl2, 1X Taq Buffer (75 mM Tris-HCl pH 8.8, 20 mM (NH4)2SO4, 0.01% Tween 20), 1 unit of Taq DNA polymerase and 250 ng genomic DNA completed to a final volume of 25 µL. PCR protocol was started with initiation denaturation for 5 minutes at 96°C followed by 35 cycles of denaturation for 30 seconds at 96°C, ligation for 30 seconds at 57°C, elongation for 1 minute at 72°C and final elongation for 10 minutes at 72°C. PCR products were run on 2.5% agarose gel electrophoresis at 110 volts and examined under UV light. Mutation presence was determined by means of the single-strand conformational polymorphism (SSCP) method. SSCP was performed with dilution of 5 µL PCR product in 5 µL loading buffer (95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol) and denaturation for 10 minutes at 98ºC. Denatured PCR products were run on 12% polyacrylamide gel (PAGE) in vertical electrophoresis for 5 hours at a current of 30 mA and subsequently stained with silver nitrate (Figure 1).

PCR and Restriction Fragment Length Polymorphism (RFLP) were used to determine the P53 codon 72 Arg/Pro gene polymorphism. PCR was performed with 0.5 nmol of each primer with;

F: 5'-TTGCCGTCCCAAGCAATGGATGA-3' and
R: 5'-TCTGGGAAGGGACAGAAGATGAC-3'

0.2 mM dNTP, 2.5 mM MgCl2, 1X Taq Buffer, 1 unit of Taq DNA polymerase and 250 ng genomic DNA completed to a final volume of 25 µL. In order to obtain the 199 bp PCR product, the protocol was started with initiation denaturation for 3 minutes at 94°C followed by 35 cycles of denaturation for 15 seconds at 94°C, ligation for 15 seconds at 68°C, elongation for 30 seconds at 72°C and final elongation for 10 minutes at 72°C. For the RFLP procedure, 5 µL PCR product and 1 µL of BstUI (Bsh1236I), the enzyme which cuts the 5'...C G↓C G...3' region were incubated for 3 hours at 60°C in 5 µL Fast

Digest Green Buffer. PCR products were run on 3% agarose gel electrophoresis at 110 volts and examined under UV light. The allele producing the 113 bp and 86 bp bands was considered Arg while the allele producing the 199 bp was considered Pro (Figure 2)[7].

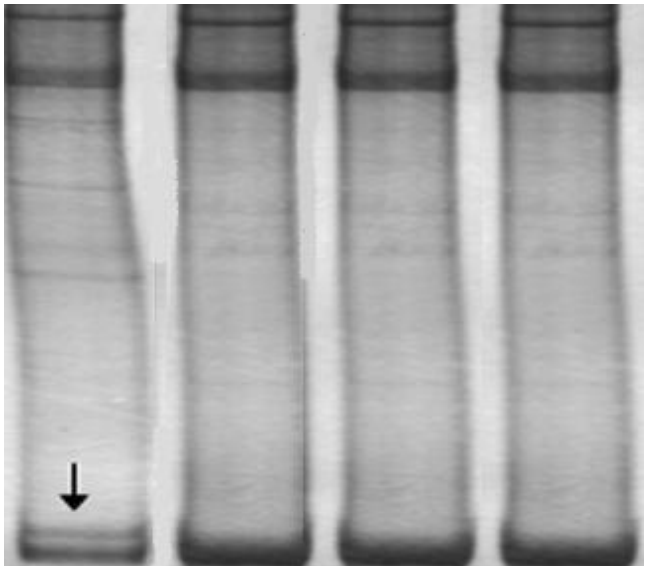

Figure 1. Single-strand conformational polymorphism with BRCA1 exon 2 samples; wild-type DNA (2, 3, 4) and mutant DNA (1).

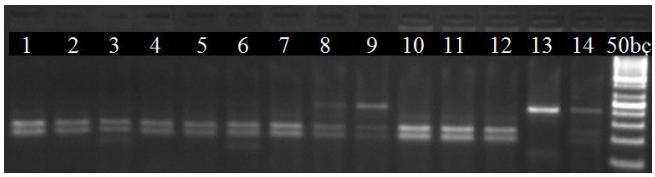

Figure 2. The run on 3% agarose gel and imaging under UV light following treatment with the BstUI restriction enzyme for the regions containing the P53 codon and 72 Arg/Pro gene polymorphism in worker-control DNAs. Genotype imaging for Arg/Arg (1, 2, 3, 4, 5, 6, 7, 10, 11 and 12), Arg/Pro (8, 9 and 14) and Pro/Pro (13) (50 bp; DNA marker).

Statistical Analysis

The results are expressed as mean ± standard deviation or percentage values. Single sample Kolmogorov-Smirnov test was used to analyze normal distribution eligibility of the values obtained from the groups. Allele frequency was calculated for both genotypes and analyzed by means of Hardy-Weinberg Equilibrium. Genotype and allele differences of the two groups were compared using the chi-square test, and odds ratios and 95% confidence intervals were calculated. P-value <0.05 was considered statistically significant. SPSS 19.0 and Stata 12.0 programs were used for statistical analyses.

Results

The limited number of cases is a limitation of the present study. Increased number of subjects would allow more significant outcomes. The exon 2 mutation of BRCA1 was detected in only 1 worker in the occupational exposure group. It should be noted that the worker was also cancer and receiving treatment. It remains unclear whether the mutation was cancer-related or a result of occupational exposure. In conclusion, BRCA1 and BRCA2 mutations were not found statistically significant among high-voltage transmission line workers in the present study (p>0.05). P53 polymorphism among the workers was also not different than the control subjects (p >0.05). However, further studies are required to clarify the underlying causes of the mutation detected in a worker included in the study group.

Discussion

The impact of electromagnetic fields (EMF) on human health has become a subject of interest for scientific researches with the increasing use of devices such as mobile phones, wireless internet, radio and TV broadcasts etc which work by electricity and therefore create EMF in environment. However, these gadgets have become indispensable in daily life. Studies on biological effects of high-voltage electric field have demonstrated that exposure to such fields alter permeability by interacting with the cellular membrane, affect Na-K pump activity, and decrease serum plasmin and cerulosplasmin levels as well as the albumin/globulin ratio. Furthermore, it has been found to increase specific group components and post-albumin levels[8]. Dasdag et al investigated the neurological and biochemical effects of long-term RF and microwave exposure in the technicians occupationally exposed. They reported that although the results found in reference range, blood parameters of EMF exposure group were found different than the control subjects. They therefore stated that EMFs may alter some blood parameters[9]. It is also reported that MW radiation may cause behavioral changes in humans[10].

Results of a study performed on people's nutritional habits working in the radio broadcasting station, TV transmitter and radio link stations showed a significant increase in lipids, carbohydrates, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and sodium intake[11]. In terms on hormones, significant increases have been observed in growth hormone ad testosterone levels in rats exposed to sinusoidal magnetic field in a dark setting[12]. Significant changes have been reported in T_3, T_4, estradiol, testosterone and progesterone levels among technicians exposed to radio-frequency and microwave radiation occupationally, and these changes have been deemed worth to be investigated[13].

Changes in serum biochemical parameters have also been observed with the effects of low-frequency radiation (such as RF and MW) in individuals who work in such fields (14). No change was seen in sperm count, p53 protein levels and serum metal ion levels except Mn^{+2} in rats exposed to extremely low-frequency magnetic field. Some studies suggest that long-term ELF-MF exposure may alter serum NO levels[15]. In another study, long term considerably low frequency magnetic field was associated with no change in oxidative and anti-oxidative processes and lipid peroxidation levels in submandibular gland; however, exposure to long-term magnetic field of 100 and 500 μT was associated with decreased catalase activity in parotid gland.[16,17] Another studies on miRNA reported that EMFs may alter some of miRNA levels in brain tissue.[18,19] It is also reported that EMFs or ELFMFs may affect DNA of some organs, cause DNA oxidation and alter the apoptotic process.[3, 4, 20, 21]

Conclusions

In light of the currently available information, it can be stated that ELFMFs or EMFs are not as innocent as they are predicted. As it is stated above the exon 2 mutation of BRCA1 was only detected in one worker in the occupational exposure group. It is not easy associate this mutation with ELFMF exposure. However, one worker with mutation in the thirty-five technician is not something to be ignored. As it is stated it remains unclear whether the mutation was cancer-related or a result of occupational exposure. Therefore the issue should be studied more extensively with the larger exposure groups. It should be noted that International agency for Research on Cancer (IARC) has still classified extremely low frequencies magnetic fields and radiofrequencies as possibly carcinogenic to human.[22]

Declaration of Interest

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