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Effect of Radiofrequency Electromagnetic Field on Human DNA

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ABSTRACT

Biological effect of radiofrequency electromagnetic radiation on the human genome has been a cause of growing concern owing to its increased application. In the present paper, effect of radiofrequency electromagnetic radiation on deoxyribonucleic acid (DNA) of the personnel working near/in radiofrequency electromagnetic radiation environment (radar operators and radio operators) has been evaluated. Monomorphic hybridisation profile was thought to be of particular interest for monitoring subtle changes in the DNA, loss or gain of sequences, or alteration in the enzyme recognition site, if any, and for direct assessment of allele length variation and allele dropout as a consequence of radiofrequency field. Such profiles were obtained using synthetic repeat oligodeoxyribonunucletide probes in conjunction with different restriction enzymes. Of the several enzymes used, *Bam*HI digest uncovered sequence modulation in one of the alleles in the region of 12-13 kb in the exposed personnel with increased frequency compared to the control individuals. This study suggests that some loci in the human DNA may be more prone to mutations arising due to radiofrequency electromagnetic radiation.

Keywords: Radiofrequency electromagnetic radiation, allele length variation, DNA typing, synthetic oligo probes, variable-repeat loci

1. INTRODUCTION

The ever-increasing presence of electromagnetic field (EMF) in the environment, from static field to microwave, has prompted studies on its effect on biological systems. Over the past decade, many reports based on *in vivo* studies on man and animals as well as *in vitro* studies on less complex biological systems, have been published[1-3]. Opinions are often divided regarding the carcinogenic potential of radiofrequency (RF) radiation and reviews of the epidemiological studies on both occupational and general public RF exposure state that there is no consistent evidence of a carcinogenic hazard. Nevertheless, a few studies do indicate positive evidence of carcinogenicity[4,5] as well as increased incidence of cancer in human beings due to RF exposure[6-11]. Mechanism by which normal cells are transformed into neoplastic ones involves structural alterations of deoxyribonucleic acid (DNA), viz, point mutation, translocation, deletion, and amplification in the somatic cells[12,13]. Downstream effect of RF

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on cellular DNA and somatic intrachromosomal recombination-inversion events have also been reported [14].

Studies on the genotoxic effect of electromagnetic field are scanty[15-18]. One possible approach to study such effects is monitoring changes in the repetitive regions of the genome wrt their modulation/alteration after exposure to electromagnetic field[19]. Recombination and other mutational events occur more frequently in the repetitive DNA than in the unique DNA sequences, which lead to a high level of genetic diversity at these loci[20]. Several of the repeated DNA sequences are distributed in a non-random fashion in the eukaryotic genome and have been classified into various subfamilies. These include, satellite structures spanning thousands of kilobases, minisatellites, and variable-number tandem repeats (VNTRs) spanning a few kilobases, and very short redundancies, ranging from 2-9 nucleotides such as (CA), (TGG) (GGAT), $(GATA)_n$ and $(GACA)_n$. Simple GATA/GACA repeats have been found to be ubiquitously present in species ranging from moths to mice and human beings[21]. GACA simple repeats have been shown to have low polymorphic information content (PIC) in human beings with a mutation rate of 0.001/fragment/gamete[22]. These repeats have been shown, both by cDNA library screening as well as by database search, to be part of transcribing sequence of many genes and have also been found to be part of several polymorphic loci as revealed by (basic local alignment search tool) BLAST search.

*A few RE and GACA-based oligo probe combinations, under high stringent conditions, were found to uncover multilocus monomorphic band pattern. The multilocus monomorphic profile was thought to be particularly helpful in assessing the effect of electromagnetic radiation, if any, on the genome because any loss or gain of band due to sequence rearrangement after radiation exposure, could be easily identified without prior knowledge of genes or its mutant alleles[19, 23]. In the present investigation, the GACA probe was therefore used as a biological end-point to ascertain if any correlation could be detected between the radiofrequency radiation exposure and enhanced level of DNA sequence modulation as a consequence of radiation effect. The DNA typing using several enzymes and GACA-based oligo probes was employed to ascertain allele length variation in DNA samples of personnel working on equipment known to emit radiofrequency radiation. Besides, biological significance of this study in the context of radiofrequency effects on human genome is highlighted.

2. PERSONNEL SELECTION

2.1 Volunteers

The personnel chosen for the study were healthy Indian male army jawans who maintained a similar kind of healthy life style and had uniform ration scale. The personnel included radar operators, radio operators, and gunners who were occupationally exposed to EMF during their entire tenure of service (Table 1). Immediately prior to this study, the personnel were also on exercise duty in the field area where they worked daily for two hours in the morning for one month on the radar or the radio signaling equipment (ANPRC 25). The gunners worked 50 m away from the radar. The carrier frequency for radar was 8.6 GHz to 9.5 GHz, amplitude modulated with average power output of 115 W. Near-field power density of the radar was approximately 1.3 W/m^2 at 10 m and the distance of the radar operator from the source was between 1-10 m. The carrier frequency for radio set was 30 MHz to 75 MHz, frequency modulated and with average power output of 15mW to 20 mW with booster. Instrument measurement of the RF field near the worker, and hence the SAR calculation, could not be done in the present experimental setup as the subjects were posted in the field area for varied period of time during their tenure (chronic exposure) and reported after completion of their exercise duties. The control subjects were also chosen from the same unit, and thus, they maintained the same kind of life style and were on the same ration scale as the workers exposed to the RF field. The control personnel worked as support staff, like administrator, cook, driver, washerman, barber, etc and were not exposed to the radiofrequency field. A few control subjects were also chosen from the general population. As per the questionnaire,

* In the earlier observations by the authors, human DNA digested with various restriction enzymes and probed with repeat-sequence oligo probes, uncovered various hybridisation profiles, viz, multilocus polymorphic hybridisation profile, multilocus monomorphic profiles, single-locus polymorphic profile, and single-locus monomorphic profile (unpublished).

Table 1. *Bam*HI profiling of DNA from personnel ID probed with GACA repeat sequence indicating presence/absence of 12-13 kb band along with the molecular weight as determined by densitometric analysis using Quantity One software (BioRad)

Personnel ID	Age	State	Type of instrument	Service (year)	Presence/absence of band (+/ –)	Mol.Wt. of the band (kb)
01	35	Maharashtra	SMF radar 8.6-9.5 GHz	14	+	12.24
02	32	Uttar Pradesh	″	13	+	12.38
03	21	Uttar Pradesh	″	4	+	12.73
06	30	Kashmir	″	12	+	11.60
07	20	Rajasthan	″	< 2	+	13.58
08	22	Punjab	″	4	–	-
11	33	Punjab	″	13	+	12.53
12	24	Uttar Pradesh	″	< 5	–	-
13	31	Tamil Nadu	″	15	–	-
17	34	Haryana	″	15	–	-
18	32	Rajasthan	″	14	(+)	13.79 (15.66)
19	23	Himachal Pradesh	″	3	(+)	14.14 (16.01)
20	23	Himachal Pradesh	″	2	+	14.03
04	31	Bihar	ANPRC 25	13	–	-
05	31	Uttar Pradesh	″	12	+	13.13
09	29	West Bengal	″	11	–	-
10	33	Uttar Pradesh	″	13	–	-
21	35	Punjab	″	14	+	13.47
22	31	Rajasthan	″	14	–	-
23	21	Uttar Pradesh	″	20 month	+	13.79
24	32	Maharashtra	″	10	–	-
28	32	Tamil Nadu	″	14	–	-
31	22	Haryana	″	< 2	–	-
14	19	Tamil Nadu	Gunner 50 m away	na	+	13.65
15	20	Uttar Pradesh	″	″	–	-
16	22	Uttar Pradesh	″	″	+	13.95
27	28	Punjab	″	″	–	-
33	29	Punjab	″	13	–	-
25	22	Madhya Pradesh	Control from unit	4	–	-
26	32	Haryana	″	14	–	-
29	25	Madhya Pradesh	″	6	–	-
30	31	Bihar	″	12	+	13.16
32	23	Uttar Pradesh	″	2	–	-
34	38	Punjab	″	13	–	-
35	26	Karnataka	″	6	–	-
36	34	Maharashtra	″	15	–	-
37	36	Uttar Pradesh	″	16	+	12.59
38	37	Rajasthan	″	15	–	-
39	24	Orissa	″	4	–	-
40	35	Uttar Pradesh	″	18	–	-
41	20	West Bengal	″	< 3	–	-
42	24	Uttar Pradesh	Civilian control	1	+	13.40
43	32	Bihar	″	7	–	-
44	29	Uttar Pradesh	″	5	–	-
45	32	Haryana	″	13	–	-
46	26	Tamil Nadu	″	8 month	[(+)]	12.83
47	26	Andhra Pradesh	″	8 month	[(+)]	13.04

na indicates that information is not available, [(+)] indicates faint band, (+) indicates band also at a higher molecular weight

most of the volunteers were non-smokers, either moderate on alchohol consumption or non-alcoholic, and had no history of any major ailment. Married personnel did not report of any abnormal pregnancy or miscarriage in their partners, or congenital malformation in the progeny. Informed consent was obtained from the volunteers prior to blood collection.

3. METHODOLOGY

3.1 DNA Analysis

DNA was isolated from 5 ml blood (drawn into heparinised vacutainers) according to the standardised method[19]. Approximately 5 µg DNA was used for digestion with different restriction enzymes[1] (*Hinf*I, *Mbo*I, *Bam*HI, *Alu*I, *Hind*III, *Kpn*I, *Eco*RI) following suppliers recommendation (New England Biolabs, USA). Digested samples were fractionated on 20 cm long 0.8 per cent agarose gel in 1 x TAE buffer over 20–21 h at 2V/cm. DNA was blotted on nylon membrane (Stratagene UV Duralon) and immobilised by UV cross-linking, following standard method.

Synthetic oligodeoxyribonucleotide repeat sequences were end-labeled with [γ^{32}-p] ATP (Amersham, UK), and hybridisation and autoradiography were conducted as reported earlier[24]. Densitometric analysis was done using Quantity One software program (BioRad). The experiments of restriction digestion and southern hybridisation were repeated thrice with each individual DNA sample, or in some instances, more than one time for conformation of band profiles of each individual.

3.1.1 *Scoring & Analysis of DNA Bands*

For statistical analysis, conventional estimates of probabilities of identities were followed[25]. Discernible bands in the range 2-23 kb were taken directly from the x-ray autoradiograms. Each band pattern (individual A) was compared with the same in the adjacent gel track (individual B). The number of bands in individual A which were clearly absent in individual B, plus those which had a comigrating counterpart of roughly similar signal in individual B were scored. Weak hybridising bands in individual A which were matching strongly hybridising fragments in individual B, were also scored. The average number (N) of different bands per individual was obtained by dividing the sum total of bands by the total number of individuals. The probability (x) that a fragment in individual A is also present in individual B was calculated independently for the control individuals and for the individuals working with microwave-based equipment using the formula

$$x = \sum D / \sum n_2$$

where D is the coefficient of band sharing and n_2 is the number of observations. Probability of identity was calculated as x^n where n is the mean number of variable bands per individual. Mean allelic frequency (q) was calculated by the formula $q = [2 \pm \sqrt{D}]/2$ where discriminant D was calculated as a value of $4 - 4x$. Percentage heterozygosity (h) was calculated as $h = 2(1-q)/(2-q)$.

4. RESULTS

Most of the enzyme-probe combinations, used in the present study, detected monomorphic profiles. No statistically significant difference in the band profile between the workers exposed to the RF field and the control individuals was observed with many of the restriction enzymes used. Some restriction digests like *Hinf*I and *Mbo*I showed moderate polymorphism among the individuals. In such cases, the probability of identity was calculated for ascertaining differences between the workers exposed to the RF field and the control individuals showing moderate polymorphism. The number of common bands, the mean number of different bands per individual, and per cent heterozygosity are shown in Table 2.

From the hybridisation result of 24 mer GACA oligos with *Hinf*I-digested DNA samples in the control individuals, the average number of bands per individual in the region of 1.5-23 kb was calculated to be 5.07 ± 1.46 and the mean band sharing between pairs of individuals was 0.804 with a probability of identity as 0.33. Mean allelic frequency (q) was 0.56 and heterozygosity (h) was 61 per cent. Amongst the personnel working with microwave-based equipment, the values obtained were almost similar as those of the control individuals. With *Mbo*I-digested DNA, the average number of bands per individual in the workers exposed to the RF field and the control individuals was found to be 5.05 ± 0.74 and 10.63

Table 2. Polymorphic information content of $(GACA)_n$ repeat oligo used in DNA profiling of controls and radiofrequency radiation-exposed workers

Sample	Enzyme	DNA size range (kb)	Mean no. of variable bands per individual (n)	No. of common bands	Mean band sharing between pairs of individual (x)	Mean allelic frequency (q)	Heterozygosity (h) (%)	Probability of identity (x^n)
Control	*Hinf* I	2-23	5.07 ± 1.460	3	0.804 ± 0.123	0.560	61	0.330
Worker	"	"	5.26 ± 1.210	3	0.850 ± 0.100	0.610	56	0.425
Control	*Mbo*I	2-23	10.63 ± 3.139	7	0.816 ± 0.112	0.571	60	0.115
worker	"	"	5.05 ± 0.740	4	0.888 ± 0.082	0.665	50	0.548

± 3.139, respectively. The mean band sharing probability in the control individuals and the individuals exposed to the RF field was calculated to be 0.816 ± 0.112 and 0.888 ± 0.082, respectively. Mean allelic frequency (q) for the alleles detected by GACA oligos was 0.57 for controls and 0.665 for the workers exposed to the RF field.

A very interesting observation was made in the hybridisation profile of *Bam*HI-digested DNA (Fig.1). In this profile, there were two common bands in the molecular weight regions of 18 kb and 7 kb in both the controls and the RF radiation-exposed workers. Additionally in the RF radiation-exposed workers, a band in the region of 12-13 kb was found to be present in 12 individuals out of the 23 individuals studied (52.17 %) (Table 1; Fig. 1). In the control individuals, this band was detected in 5 out of 19 individuals (26.31 %). Additionally in two RF-exposed workers, (personnel ID 18 and 19), another band at a higher molecular weight region (15-16 kb) was also detected, apart from the 12-13 kb band. In two control cases, (personnel ID 46 and 47), however, this band was of relatively weak intensity, implying copy number variation. In the case of gunners, 2 out of 5 individuals showed the presence of the 12-13 kb band, although at this stage, the possibility of the presence of confounding factors wrt the gunners is not ruled out.

5. DISCUSSION

The GACA repeat is a highly conserved sequence in eukaryote and distributed ubiquitously in the genome[21]. In the present study, it was sought to determine whether the GACA-containing repeat loci were affected in personnel working on and handling the radiofrequency radiation equipment.

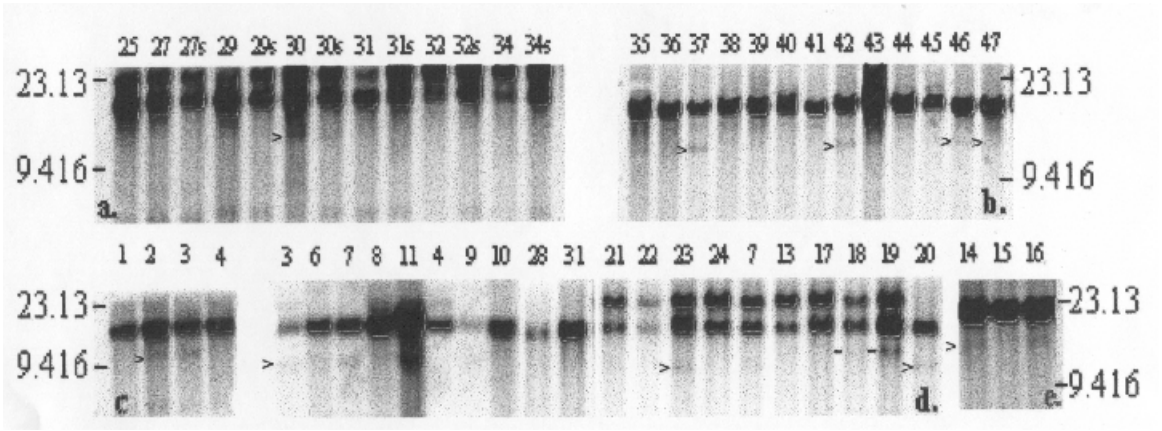

Figure 1. Representative hybridisation profile of human lymphocyte DNA digested with *Bam*HI and probed with $(GACA)_6$ repeat oligo sequence. a and b are control DNA and c-e are DNA from theworkers. Numerals above the lanes correspond to personnel identification number (ID) as given in column 1 of Table 1. Markings on the left and the right represent molecular weight band (kb). Note the presence of 12-13 kb band (marked >) in control samples (30, 37, 42, 46 and 47) and workers (1, 2, 3, 6, 7,11,21,23, 18, 19, 20, 14 and 16). Also note the higher molecular weight band (15-16 kb) in personnel ID numbers 18 and 19. Profile from ID numbers 3 and 7 are shown in replicate from two independent runs. Numerals marked with s represent semen DNA.

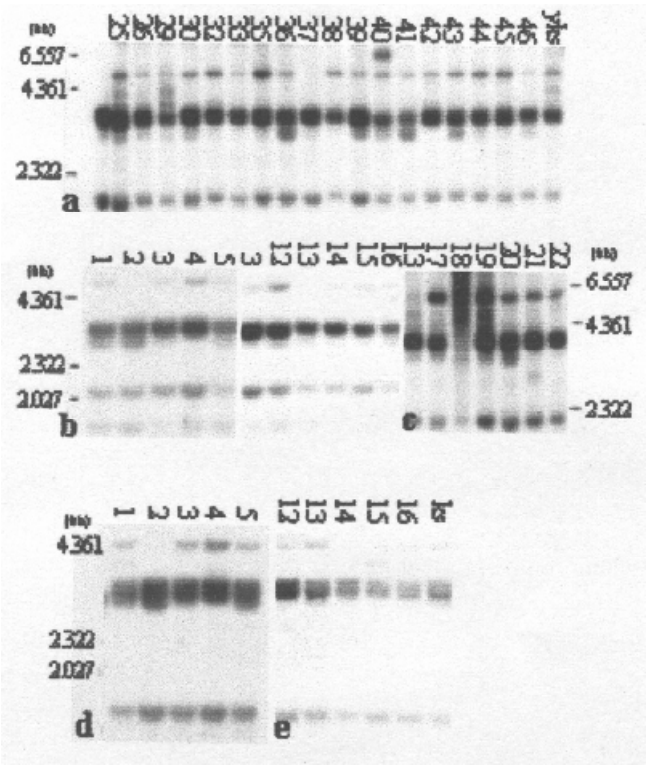

Figure 2. Representative hybridisation profile of human DNA digested with *Hinf* I (a, b and c) and *Mbo*I (d and e) in the region of 7.0-0.5 kb showing the presence of moderate polymorphism: (a) represents profile of control DNA and (b-e) are profiles obtained from workers. Numerals above the lanes correspond to personnel ID numbers as given in Table 1. Markings on the left and the right represent molecular weight (kb).

The most important observation was made in the *Bam*HI-digested DNA in the RF radiation-exposed workers compared to the control individuals. A 12-13 kb allelle was detected in 52.17 per cent of the exposed personnel (n = 23), while in controls (n = 19), it was detected only in 26.31 per cent of the cases.

Although this observation does not provide a conclusive evidence that the appearance of the 12-13 kb band is due to radiofrequency radiation, but a 2-fold increased frequency of occurrence of this band in the RF radiation-exposed individuals vis-à-vis control individuals seems possible. Since this band is present in 26 per cent of the control subjects also, it is probable that these sequences are present in the population with copy number variation; in the control group individuals who do not show the presence of the band, the copy number of these repeat sequences may be insufficient to form a distinct band. Occupational exposure to the radiofrequency field may have led to the amplification of these tandem repeats in the RF radiation-exposed workers, generating more copies of the GACA sequences in this particular region.

If the RF radiation-exposed workers are categorised into different groups, then it is observed that the 12-13 kb band is present in 9 out of 13 workers working on radar (69 %) and 4 out of 10 radio operators (40 %). Interestingly, DNA rearrangement was also observed in the mice genome after microwave exposure (frequency 2.45 GHz, power density 1mW/cm^2) in the GACA-containing repeat loci in a 7.7 kb allele identified by *Hinf*I digestion[18].

Although, till now it is not known whether exposure to a mutagenic agent or a specific class of mutagen increases the mutation rate in the region of these tandem repeats, it is known that stress induces amplification by extra replication of DNA segments in the non-coding repeat sequences[26]. Family study would be of interest to test whether the 12-13 kb *Bam*HI band is uncovered in the parents of the RF radiation-exposed workers or whether the same has occurred due to amplification of these sequences due to non-specific stress phenomenon of electromagnetic field. Another aspect to be studied would be ethnicity variation, if any.

cDNA library screening with GACA probe has shown these sequences to be part of several transcribing genes. It may also be noted that BLAST search of Gene Bank database substantiates the transcribing status of the GACA repeats. However in the present study, it is refrained to comment whether the locus in the range 12-13 kb represents a transcribing sequence or not. Nevertheless, this locus seems to be affected by radiofrequency radiation and shows sequence modulation noticeably higher than the usual sporadic-level mutation.

The GACA repeat motifs have also been suggested to be associated with nucleolar organising regions (NORs) in human beings probably as a consequence of accumulation of these motifs in the spacer regions

between the ribosomal RNA genes[27]. It would be interesting as well as informative to analyse such regions for the alterations or endoreduplication of the sequences adjacent to nucleolar organising regions carrying GACA repeat motifs to monitor the effects of electromagnetic field.

Biological effects of electromagnetic fields in the frequency range 300 Hz to 300 GHz have been reviewed by the expert committees of various countries[1,2,28]. A basic restriction for the specific absorption rate of 0.4 W kg^{-1} for the RF radiation-exposed workers has been proposed based on the observation that exposure for more than 20 min to a specific absorption rate of 0.4 W kg^{-1} results in a rise in the body temperature by approximately 1 ^{o}C. Although the human body can tolerate such an increase in temperature[28], it is uncertain whether a long-lasting elevation of the body temperature increases the risk of adverse effects. In the present experimental setup, the specific absorption rate and the electromagnetic radiation dose reaching the workers could not be determined because they were in the field area prior to this study. However, the current findings on sequence modulation in one of the 12-13 kb alleles in the RF radiation-exposed workers would prove to be an entry point for further probing the role of electromagnetic radiation on the sequence modulation in the human genome and its likely biological and genetic fallout.

Many investigators have used human lymphocytes to study the biological effect of electromagnetic field[29]. Evidences of mutational activity in white blood cells have been reported from personnel who were accidentally exposed to the electromagnetic radiation while repairing microwave devices[16,28] though such effect has not been seen in normal lymphocytes[30] exposed to low frequency, low energy pulsed electromagnetic field[31]. Daily[32] reported a statistically significant increase in immature red blood cells among the workers exposed to radar when the same was first identified as a health risk.

Goldoni[33] compared the hematological findings in 25 male air traffic control technicians exposed to radar (radar-exposed technicians) with those 10 electronic technicians working at a distance from the microwave source and reported that radar-exposed workers had significantly lower levels of leukocytes and red blood cells than the technicians working on the microwave devices. In a follow up study of 49 radar-exposed technicians, thrombocyte and leucocyte counts decreased though these stayed within the normal limits[11].

A series of studies from Croatia and Italy have also demonstrated that radar exposures are mutagenic both *in vitro* and *in vivo*[11]. In the light of these reports, it may be logical to attribute enhanced occurrence of 12-13 kb band allele in the RF radiation-exposed workers to the electromagnetic field exposure. The present study suggests that the region 12-13 kb is prone to mutation/modulation, which is enhanced to more than the normal sporadic level in response to electromagnetic radiation.

6. CONCLUSION

In conclusion, the present data indicates DNA modulation in the lymphocytes of workers working on the radar and the workers exposed to radiofrequency electromagnetic field, supporting the hypothesis that radiofrequency radiation affects DNA. It remains to be seen whether in the long run, such effects are deleterious or beneficial, reversible or permanent and/or heritable.

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Contributors

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