Research Article

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PVC WORKERS AND THEIR CYTOGENETIC EFFECTS

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Abstract: The industry categories identified vinyl chloride is the chemical used in synthesis for the plastics industry. It is an industrial intermediate chemical that is converted to polyvinyl chloride (PVC) polymer and associated copolymers. It is also used for production of chlorinated solvents, primarily 1, 1, 1-trichloroethane. PVC is used in most industrial sectors and accounts for around one fifth of plastic material usage worldwide. Vinyl chloride liquid is fed to polymerization reactors where it is converted from a monomer to a polymer PVC. The final product of the polymerization process is PVC in either a flake or pellet form. The manufacturing plastics often create large quantities of toxic chemical pollutants such as dioxin, hydrochloric acid, and vinyl chloride. This poses a severe health risks to humans during the PVC life cycle. These toxins can produce severe illness like cancer, diabetes, neurological damage, reproductive and birth defects. Dioxin is a persistent Organic Pollutant (POP), these are chemical substances that persist in the environment, bio-accumulate through the food chain, and pose a risk of causing adverse effects to human health and the environment. The focal aim of the present study was to identify the genetic effect of PVC workers who were chronically exposed to PVC manufacturing industry in Coimbatore & Tirupur districts. The exposed workers were categorized based on the duration of exposure to PVC chemicals. Moreover, both the exposed subjects and controls were divided according to age wise manner. The groups were divided into four categories namely group. To the best of our knowledge; this is the first kind of study in PVC industry workers in Coimbatore and Tirupur district. The present investigation will definitely gain the consequence of the better resolution to prevent the effects from chemicals entering into the occupationally exposed workers with protection and prevention strategies.

Keyword: Vinyl chloride, Chromosomes, Genetic effect, Cell and Environment

INTRODUCTION

A Chromosome is an organized structure of DNA and protein found in cells. The word chromosome comes from Greek word (chromo, color) and (soma, body) due to their property of staining by particular dyes. It is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions. Chromosomes vary widely between different organisms. In the nucleus of each cell, the DNA molecule is packaged into thread-like structures called chromosomes. Each chromosome is made up of DNA tightly coiled surrounded by the proteins called histones that support its structure. Each chromosome has a constriction point called the centromere, which divides the chromosome into two sections, or "arms". The DNA molecule may be circular or linear, and can be composed of 100,000 to 10,000,000,000 nucleotides in a long chain. In eukaryotes, nuclear chromosomes are packaged by proteins into a condensed structure called chromatin. This allows the very long DNA molecules to fit into the cell nucleus. The structure of chromosomes and chromatin varies through the cell cycle. Chromosomes are the essential unit for cell division and must be replicated and passed successfully to the daughter cells so as to ensure the genetic diversity and survival of their progeny.

Polyvinyl chloride, (IUPAC Poly (chloroethanediyl)) commonly abbreviated PVC, is a thermoplastic polymer. It is a vinyl polymer constructed of repeating vinyl groups (ethenyls) having one of their hydrogen replaced with a chloride group.This is a plastic that has the following chemical formula: $CH_2=CHCl$. After the First World War, there were a boom in new forms of plastics due to the improvements in the chemical technology sector, including "polystyrene (PS)" and "polyvinyl chloride (PVC)", developed by the I.G. Farben company of Germany. Plastic covers a wide range of synthetic or semi-synthetic polymerization products (i.e. long-chain carbon-based "organic" molecules) which refer to the semi-liquid state .They are malleable with the property of plasticity. Polyvinyl chloride (PVC) was one of the first plastics manufactured in 1930. Today it is the second most commonly-used plastic in the world, which is estimated that 59 billion pounds were produced globally in 2002. Although PVC swells or dissolves in aromatic hydrocarbons, ketones, and cyclic ethers, PVC is hard to dissolve in other organic solvents. Taking advantage of this characteristic, PVC is used in exhaust gas ducts, sheets used in construction, bottles, tubes and hoses. The workers in VCM manufacture and PVC polymerization and fabrication, the route of exposure is by inhalation. Much of the animal carcinogenicity data are based on inhalation exposure and the human epidemiology is predominantly of populations exposed occupationally by inhalation. Thus the present study is for the assessment of the risk factors and the quantitative risk of inhalation exposure.

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Literature Review:

Vinyl chloride monomer (VCM), mainly used as a raw material for the manufacturing of polyvinyl chloride (PVC) resins, was considered to have low toxicity until 1949 when workers with hepatitis were noticed VCM was suspected to be a potential carcinogen since 1970s due to several animal and epidemiological studies. The mechanism of the VCM carcinogenic effect is usually thought to be an epoxide generation, which causes DNA alkylation (Bolt *et al.,* 1981). Under the condition of heavy burden from excessive VCM exposure, repair defect ensures lesions which may be expressed. These ranged from DNA-base-pair adduct, substitution addition, deletion, DNA strand separation or sister chromatid exchange (SCE) to chromosomal or gene abnormality (Brusick, 1987) Methods of detecting DNA damage repair include detection of damaged bases, detection of strand breaks, and detection of incorporation of new bases (Cleaver, 1978) Single strand DNA exists in normal blood lymphocyte and repairs continually. In one study, it was shown that after 24 h of exposure of mice to VCM, the level of SSB is correlated with VCM concentration, and after 20 h post-exposure, 80% of DNA damage had been repaired (Walls *et al.,* 1984). Vinyl chloride monomer (VCM) is one of the important industrial chemicals (Ghissassi *et al.,* 1998) known to be potentially hazardous for those exposed in the workplace as well as for the non-worker population residing near PVC chemical plants.

The over expression of circulating mutant (Smith *et al.,* 1998) p53 protein was associated with increasing VCM exposure among French PVC workers, a similar finding being reported for Taiwanese PVC workers (Luo *et al.,* 1999). The toxicity of vinyl chloride, released in the production of PVC, is well-characterized (Wong *et al.,* 1991) California Environmental Protection Agency (Cal-EPA, 2006) International Programme on Chemical Safety (Zdzienicka *et al.,* 1992). In 1987, the International Agency for Research on Cancer (IARC) classified it as a Group 1 known human carcinogen, based on a substantial body of animal and human studies. In animal studies, vinyl chloride has been shown to be mutagenic, carcinogenic, and have adverse reproductive and developmental effects (Cappelli *et al.,* 1997). Similarly, carcinogenic, reproductive, and developmental effects have been documented in epidemiologic studies of workers occupationally exposed to vinyl chloride (Easter and Von Burg 1994). In animals, vinyl chloride exposures have been associated with an excess number of cancers, including those of the mammary gland. In humans, the evidence for carcinogenicity is strongest and most consistent for liver angiosarcoma, with more limited evidence for brain cancer, lung cancer, and lymphoma. Due to the less number of women working in occupational settings with PVC exposures, it has not been possible to fully assess the risk of breast cancer associated with vinyl chloride exposures in women.

Active metabolites of VCM are known to form etheno-adenosine adducts in DNA, these adducts possibly resulting in A to T transversions (Yu-lan Qiu *et al.,* 2012) with such A to T transversions having been found in the DNA from some ASL cases for VCM-exposed workers (Hollstein *et al.,* 1994). Workers from the high VCM-exposure group experienced a greater chance of developing DNA damage than from the low VCM exposure group, and we speculate that an increased mutation frequency for the p53 gene among XRCC1 Gln-Gln carriers might additionally prompt the persistence of DNA damage. Interestingly, those individuals who demonstrated with more susceptible genotypes of XRCC1, CYP2E1, ALDH2, and GSTT1 were more likely to experience p53 over expression. CYP2E1 and GSTT1 are involved in the activation of VCM, and ALDH2 acts as detoxifying enzymes for the reactive metabolites of VCM, whereas XRCC1 is involved in the subsequent DNA-repair process. This indicates that each susceptible genotype may not generate a significant risk for p53 over expression; however, when they are combined together, a more prominent risk may develop. It seems that subjects who carry susceptible genotypes of metabolic and/or DNA repair traits are more likely to express p53 mutation when they are exposed to VCM regardless of high or low VCM cumulative dose. The effects on the genes XRCC1, CYP2E1, ALDH2, and GSTT1 indicates that the chromosomes 17, 9, 10 & 12 undergo changes due to the PVC exposure in the workers. The mutations in these chromosomes due to PVC lead to various cytogenetic and other effects (Smith *et al.,* 1998).

MATERIAL AND METHODS

Sample Collection:

The blood samples of about 2ml were collected from the individuals, working in PVC industries in Coimbatore & Tirupur districts. Samples were collected from all persons of the mentioned ages and gender and were then categorized. Venous blood samples (5 ml) were drawn in heparinised syringes from each subject. Totally 36 samples was taken which includes 18 experimentals and 18 controls. The exposed subjects and controls were categorized based on the gender and age into four groups namely group I (age 20 - 35), group II (age 36 – 45), group III (age 46 – 55) and group IV (above 55age). The controls were healthy individuals residing in that same area and they were also categorized as same as the experimental. Moreover questionnaires were prepared and the questions were relating to lifestyle, consumption habits such as smoking, alcohol intake, medication, diagnostic tests or previous occupational exposures to chemicals. The questionnaires are of distributed to all donors and make them to fill all related information.

Establishment of the culture:

About 2.0 ml of venous blood from the experimental subject was drawn into a sterile heparinized syringe and 0.5 ml of the blood (about 30 drops) was inoculated under aseptic conditions into a culture vial containing 5.0 ml of culture medium, 1.0 ml of AB serum and 0.2 ml of PHA. The cultures were incubated at 37°C for a period of 72 hrs and were shaken periodically twice a day in order to facilitate proper mixing of the medium and cells in culture.

Harvesting of culture:

The dividing cells were arrested at the metaphase stage by adding 0.05 ml of colchicine solution (0.01 %) at 30 minutes before harvesting the culture. The contents of the vials were centrifuged at 1000 rpm for 20 minutes at the end of colchicine treatment. The supernatant was discarded and 6 ml of pre-warmed hypotonic solution (0.075 M KCl) was added to the test tube after disturbing the cell button. The contents of the test tubes were incubated for 7 minutes. After incubation, 1 ml of freshly prepared fixative (Methanol and Glacial Acetic acid (3:1 v/v) was added and centrifuged at 1000 rpm for 10 minutes. Later the supernatant was discarded and two or three chances of the fixative were given to obtain a colourless cell pellet.

Chromosomal analysis:

Fifty well spread metaphase plates of each subject were screened under oil immersion lens of the optical microscope and selected metaphases were photographed. For the micronucleus assay, initially 4.5 ml of RPMI 1640 with L-glutamine was added to the culture vial of 30ml capacity. Followed by the addition of 1ml of fetal bovine serum, 0.1ml of phyto-hemagglutinin was added and finally 0.5ml of blood. This mixture was incubated at 37°C for about 72 hours. After 44 hours cytochalasin B was added at a final concentration of 3µg/ml. Before 45 minutes of culture duration, the spindle inhibitor 0.01% colchicines was added to the culture and incubated further. After the completion of 72 hours, the contents of the vial was transferred to a new, sterile and graduated polypropylene centrifuge tube and centrifuge at 1,500 RPM for 5 minutes. The resulting supernatant was discarded using a sterile Pasteur pipette. The remaining was vortexed gently to mix well. Then the contents were re-suspended in 0.075M KCl, pre-warmed to 37°C and left it undisturbed for a period of 15 minutes. The contents were centrifuged at 1,500 RPM and the supernatant was discarded leaving 0.5 to 1ml. The cells were re-suspended in 5ml of ice cold fixative (contained 3 parts methanol and 1 part glacial acetic acid). The fixative was added in a manner that first 1ml drop by drop, once the preparation turned black, remaining volume of fixative was added in normal manner. The contents were kept in a deep freezer for 1 hour. Then brought out and centrifuged. The supernatant was discarded and the preparation was given two more fixative changes. Finally, it was stored at -20°C before slide preparation. Before making slides, the fixative was discarded after centrifugation and re suspended in a small volume of freshly prepared fixative. In the preparation of slides, first the slides were cleaned using soap solution, washed in running tap water and kept in a beaker filled with distilled water. Before dropping cell suspension over the slide, slides were wiped with a small amount of cold fixative and the cell suspension dropped and dried over a hot plate maintained at 40°C. The slides were stained in a horizontal staining rack, contained 2% Giemsa solution made in 0.025M phosphate buffer pH 6.8 for 5 to 8 minutes. After that, the slides were picked out and gently washed with distilled water to remove the excess stain. In this way, the prepared slides were scanned under an oil immersion microscope of 100X magnification for the scoring of micronucleus.

RESULTS

The result of chromosomal alteration and micronucleus analysis of polyvinylchloride workers based on the age and life style factor were interpreted. Table 1 depicts the characteristic features of the experiments and controls including the divided groups, affected diseases & life style factors such as alcohol consumption and smoking and percentage were calculated for each group. Table 2 depicts the age and life style factors of control samples and their chromosomal effects and MN analysis. The control samples were also categorized into 4 groups as the same as experimental groups. The results depicted the fact that, as the age increases the life style factors are affected, it leads to chromosomal alterations and other defects in the humans. Here the results show that group III (age 46 – age 55), and group IV (above 55 ages) show greater chromosomal alterations when compared to group I & II. But the results of the control samples showed lesser values when compared to the experimental groups.

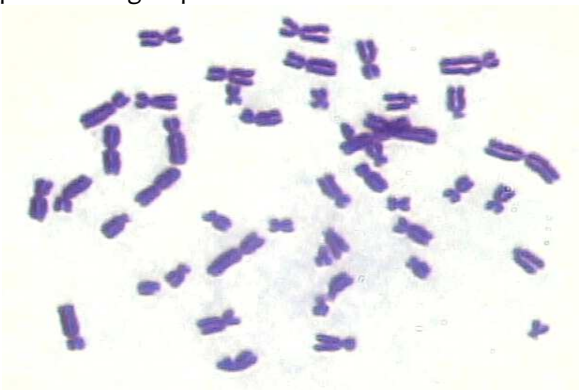

Plate.1: Control Subjects

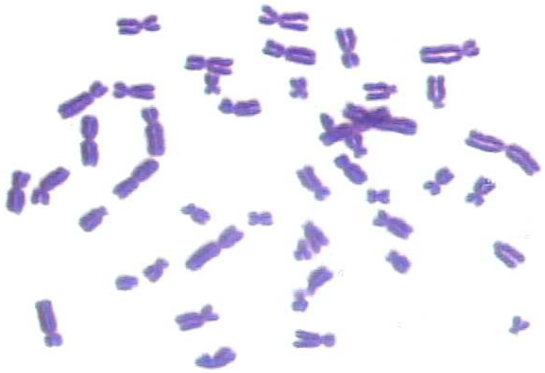

Plate.2: PVC Exposed Subjects showing Chromosome type aberration and Chromatide type aberration

The results could be concluded that life style factors have great influence on the chromosomal alterations. Also as the age increases, the years of exposure also increases which ultimately leads to the chromosomal defects and other effects. The chromosomal defects are predominant among the older groups when compared to the younger groups. Similarly, wide variations were observed between the control and experimental samples as shown in plates 1 and 2 in each analysis. Also the PVC workers are prone to liver defects and cancer than the other diseases.

DISCUSSION

Plastic covers a wide range of synthetic or semi-synthetic polymerization products (i.e. long-chain carbon-based "organic" molecules) which refers to the fact that in their semi-liquid state they are malleable, or have the property of plasticity. Vinyl chloride monomer (VCM), more properly named monochlorethane, is a colourless gas normally handled under pressure as a liquid which boils at - 14°C at normal pressure. This is the PVC from which pipes are made, and PVC pipe is everywhere. Vinyl chloride (CH_2=CHCl), also known as chloroethylene, is most often obtained by reacting ethylene with oxygen and hydrogen chloride over a copper catalyst. It is a toxic and carcinogenic gas that is handled under special protective procedures. Annual growth of the world PVC consumption is around 5%. In developing countries PVC consumption per capita is 1 kg and less than one tenth of those of developed countries. PVC is the essential material for construction of infrastructure in developing countries.

Cytogenetic studies were performed on 39 workers from a PVC plant in 1974. 16 healthy men without any connection with the plant were chosen as controls. The cytogenetic study was repeated for 37 of the 39 workers 2--2.5 years later. During this time interval the workers had only had a minimal exposure to VCM. This repeated study was performed with 32 matched controls from the office employees in the factory. Breaks, gaps and stable rearrangements were scored in 100 metaphases per person from 48-h lymphocyte cultures. The mean chromosome-breakage frequency for the workers (3.41%) was significantly higher than for the controls (1.79%) in the first investigation. In the repeated study no difference was found in mean chromosome-breakage frequency between the workers and their matched controls. Neither was there any difference between these breakage frequencies and the breakage frequency for the previous control group. These results might indicate a relationship between the reduction in exposure to VCM and the normalized chromosome breakage frequency. Sister-chromatid exchanges were studied for 16 workers with matched controls in the repeated study. A mean of 7.6 SCEs per cell was found for both workers and controls. Bone-marrow samples from 4 workers were studied in the first investigation. The mean chromosome-breakage frequency was higher in the bone marrows (4.2%) than that reported for normal bone marrows (0.2, 0.4, 1.7%), and higher than for the corresponding lymphocyte cultures (Jones *et al.,* 1988).

In the present investigation, the results of the chromosomal defects and MN analysis of the samples based on the age and life style factors were analyzed. Though the values of the control samples were lower than the experimental samples, they also showed the fact that the older groups (group III & IV) are mostly affected when compared to the younger groups (group I & II).

In the present study, Chromosomal alterations were frequently observed in chromosome 10, 12, 17 and 19. The tumour suppressor gene 17 is adversely affected and mutations in it cause the cytogenetic effects in the workers leading to cancers and other effects in them. Several major epidemiological studies on workers exposed to VCM have been reported (Yu-lan Qiu *et al.,* 2012). The organs that have been associated with higher incidences of cancer in workers exposed to VCM are the liver, lung and brain. The standardized mortality ratio of cancer in lymphomas of the buccal cavity and pharynx, cancers of the lymphatic and cardiovascular systems has been reported in few studies (Jones *et al.,* 1988). The analysis of cancer in the respiratory system is often confounded by smoking, making quantitative analysis of the contribution of VCM difficult (Belliveau and Lester 2004).

Individuals who demonstrated with more susceptible genotypes of XRCC1, CYP2E1, ALDH2, and GSTT1 were more likely to experience p53 over expression. CYP2E1 and GSTT1 are involved in the activation of VCM, and ALDH2 acts as detoxifying enzymes for the reactive metabolites of VCM, whereas XRCC1 is involved in the subsequent DNA-repair process (Fucid *et al.,* 1990). This indicates that each susceptible genotype may not generate a significant

risk for p53 over expression; however, when they are combined together, a more prominent risk may develop. It seems that subjects who carry susceptible genotypes of metabolic and/or DNA repair traits are more likely to express p53 mutation when they are exposed to VCM regardless of high or low VCM cumulative dose.

Table 1: Characteristics of the study population

S.No	Particulars	Total	%
1	**Exp**	18	100%
	Cont.	18	
2	**Groups**		
	G – I	2	11.11%
	G – II	4	22.22%
	G – III	5	27.77%
	G – IV	7	38.88%
3	**PVC Diseases (Exp)**		
	CNS - Yes	4	22.22%
	No	14	77.77%
	RE – Yes	3	16.66%
	No	15	83.33%
	LE – Yes	10	55.55%
	No	8	44.44%
4	**Smoking Status Exp and Cont**		
	Exp - Yes	13	72.22%
	No	5	27.77%
	Con - Yes	7	38.88%
	No	11	61.11%
5	**Alcohol Status Exp and Cont**		
	Exp – Yes	4	22.22%
	No	14	77.77%
	Cont - Yes	7	38.88%
	No	11	61.11%

Table.2: The Mean and SD values of total CA and MN of control and experimental samples taken from PVC workers.

Particulars		CA		Total CA	Total MN
		Major	Minor		
EXPT	Group I	0.15 ± 0.69	0.5 ± 0.70	1.33±0.57	0.5±0.57
	Group II	0.25 ± 0.5	0.5 ± 0.57	1.25±0.95	0.8±0.841
	Group III	1.2 ± 0.83	0.6 ± 0.54	2.0±1.0	1.0±1.41
	Group IV	1.5 ± 2.12	0.57 ± 0.78	1.85±0.89	1.5±1.90
CONT	Group I	0.25±05	0.5±0.58	0.5±0.577	0.25±0.5
	Group II	0.2±0.44	0.4±0.54	0.6±0.54	0.4±0.54
	Group III	0.4±0.54	0.4±0.54	1.0±0.70	0.6±0.54
	Group IV	0.4±0.89	0.4±0.56	1.4±1.14	0.6±0.89

EXPT – Experimental
CONT – control samples
CA – Chromosomal alteration
MN – Micro Nucleus
RE – Reproductive effects
LE – Liver effects

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