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CHROMOSOMAL ABERRATIONS AND OXIDATIVE STRESS INDICATORS IN PAINTERS, IS POWDER COATING SAFER?

By

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Abstract:

Background: There are different and diverse groups of paints, the potential toxicity of which depends on the types of pigments, resins and solvents used in their manufacture. Numerous recent evidences support the role of oxidative stress in the pathophysiology of genotoxicity induced by the exposure to solvent based paints. Indeed powder coatings were considered as safer than other types of paintings. Recently however health problems when handling or using a powder coating may arise through exposure to hazardous substances, which may be contained in the coatings, or to the powder coating itself. **Objectives:** to evaluate the genotoxic effects of occupational exposure to paints and the possible role of oxidative stress in the pathogenesis of chromosomal aberrations, and to compare these effects between workers exposed to solvent based paints and workers exposed to powder coatings (dry paints) which are considered as a safer alternative to solvent based paints. **Subjects:** The study population consisted of 65 males divided into 34 exposed and 31 control groups. The exposed group was comprised of all workers exposed to paint in the painting unit of a small scale factory in Cairo involved in furniture production and painting. The exposed workers were further subdivided into two subgroups, group 1 (n=23) comprised workers occupationally exposed to solvent based paints, and group 2 (n=11) comprised workers exposed to dry powder coating type of painting. **Methods:** every individual was subjected to detailed occupational and medical history, cytogenetic chromosomal analysis and laboratory investigations to evaluate the level of oxidative stress indicators, malondialdehyde

(MDA), Peroxynitrile (PN) and Total Sulphydril Groups (SHG). **Results:** The chromosomal aberrations and oxidative stress (higher MDA and PN and lower SHG) were significantly higher in the painters group compared to the control. The mean level of all aberrations except separation was highest in group 1 (solvent based painters) compared to group 2 (powder dry painters) and control group, and the difference was highly significant between group 1 and group 2 for all aberrations. The mean level of aberrations was higher in group 2 compared to the control group, but the difference was not significant for all except for break/ gap type aberrations. Oxidative stress was evident in group 1compared to group2 and the control group. On the other hand, oxidative stress was more evident in group 2 compared to the control and the difference was statistically significant. **Conclusion:** Our study confirms other studies that claim the role of oxidative stress in the pathophysiology of genotoxicity in painters especially those exposed to solvent based paints. The higher level in the chromosonmal aberrations and oxidative stress indicators in the dry powder coating exposed workers compared to the control, indeed raises suspicion that powder coatings is a "Safer Alternative to Solvent Based Paints". Adopting safe working procedures will definitely reduce the risks to a minimum.

Key words: solvent-based paints, powder coatings, genotoxicity, oxidative stress, peroxinitrile, total sulphydril groups

Introduction:

Paint is a generic name for a number of different products, and its potential toxicity depends on the types of pigments, resins, and solvents used in its manufacture. One of the major groups of paints is the solvent-based paints, in which the solvent is usually petroleum-based and organic, such as toluene or xylene. And the second group is powder coating (dry paints). This is typically produced by blending and extruding together resins, curing agents, pigments and additives. The resultant matrix is ground into fine discrete particles (Kirk-Othmer, 2006).

Solvent based paints were, for generations, the only available surface coating for industrial use on surfaces. Chemistry and creativity, however, have yielded several other options in recent years. First among these is a dry-application known as powder coat. Powder coating is a type of coating that is applied as a free-flowing, dry powder. The main difference between a conventional solvent- based paint and a powder coating is that the powder coating does not require a solvent to keep the binder and filler parts in a liquid suspension form. The coating is typically applied electrostatically and is then cured under heat to allow it to flow and form a

"skin". The powder may be a thermoplastic or a thermosetpolymer. It is usually used to create a hard finish that is tougher than conventional paint. Powder coating is mainly used for coating of metals, such as "whiteware", aluminium extrusions, and automobile and bicycle part (White ,2004)

Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells (Scélo et al., 2009).

The low molecular weight thiol, glutathione, and "reactive" protein sulfhydryls (exposed cysteines in many proteins) are primary participants in cellular anti-oxidant systems. Reactive protein sulfhydryls are abundant in both soluble proteins and in membrane-bound proteins, they are important antioxidants in the detoxification of xenobiotics, carcinogens, free radicals and maintenance of immune functions (Unsal et al,, 2005).

Simultaneous generation of nitric oxide (NO) and superoxide favors the production of a toxic reaction product, peroxynitrite anion (ONOO_). Peroxynitrite reported activities include a rapid oxidation of sulfhydryl groups and thioethers, as well as nitration and hydroxylation of aromatic compounds, including tyrosine, tryptophan and guanine (Greenacre and Ischiropoulos, 2001). The reaction of peroxynitrite with lipids leads to peroxidation (malondialdehyde and conjugated diene formation) and formation of nitrito-, nitro-, nitrosoperoxo- and/or nitrated lipid oxidation adducts (Szabo, 2003).

It is important to note that peroxynitrite can inhibit superoxide dismutase glutaredoxin and other antioxidant molecules and systems. Peroxynitrite-mediated depletion of one of the key cellular antioxidants, glutathione can lead to positive feedback cycles of intracellular oxidant generation and exacerbation of the oxidative cellular injury (Aykac-Toker et al., 2001). An important interaction of peroxynitrite occurs with nucleic acids, Peroxynitrite-induced DNA single strand breakage.

The process of lipid peroxidation is one of the oxidative conversion of polyunsaturated fatty acids to several products including malondialdehyde (MDA) and lipid peroxides. The products of lipid peroxidation are easily detected in blood plasma and urine, and have been used as a measure of oxidative stress. The most commonly measured product is malondialdehyde(Laura et al.,2003).

Malondialdehydeis a potentially important contributor to DNA damage and mutation. MDA is mutagenic in bacterial and mammalian cell assays, and it is carcinogenic in rats. The maximum increase in mutation frequency observed after MDA treatment was 15-fold above background levels (Routledgeet al.,1993).

Recent evidences support the role of oxidative stress in the pathophysiology of Genotoxicity induced by the exposure to solvent based paints (Karagözler et al, 2002, Khan et al., 2010 and Moro et al., 2010). Powder coatings were considered relatively safer than other types of paintings. Recently however health problems when handling or using a powder coating may arise through exposure to hazardous substances, which may be contained in the coatings, or to the powder coating itself. Hazardous products of degradation may be formed during the burning off of powder coating.

Triglycidylisocyanurate (TGIC) is a triepoxy compound used as a cross-linking agent in powder coatings used in the metal finishing and furniture industry (Allmaras, 2003). This chemical could give rise to adverse health effects. Recent animal toxicity studies indicated a potential for TGIC to cause genetic damage. The studies raised concern that TGIC could be a human carcinogen and mutagen and could have adverse reproductive effects. Indeed results of the Ames tests indicate that TGIC is a direct-acting mutagen but also with weak mutagenic potential (HSE, 2000).

Aim of this study:

The first aim of this work is to evaluate the genotoxic effects of occupational exposure to paints and the possible role of oxidative stress in the pathogenesis of chromosomal aberrations.The second aim of this work is to compare these effects between workers exposed to solvent based paints and workers exposed to powder coatings (dry paints) which are considered as a safer alternative to solvent based paints

Subjects:

The study population consisted of 65 males divided into 34 exposed and 31 control groups.

The exposed group was comprised of all workers exposed to paint in the painting unit of a small scale factory in Cairo involved in furniture production and painting. The exposed workers age ranged (24 - 59 years) with a mean 41.91± 10.64 years. As for the duration of work of the exposed workers, mean 20.89±10.15 years. The exposed workers were further subdivided into two subgroups, group 1 (n=23) comprised workers occupationally exposed to solvent based paints, and group

2 (n=11) comprised workers involved in the application of powder coating (dry painting).

Mean age of group1 was 40.13±10.92 and duration of work 22.30±8.78. The mean age of group 2 was 45.64 ±9.42 and duration of work 17.91 ±12.48.

The exposed workers did not use the personal protective equipment on regular basis. They worked for 12 hours a day, 6 days a week.

The control group is composed of 31 workers involved in administrative and office work and never worked in paints and not exposed to genotoxic chemicals, the control subjects were chosen so that to match the exposed group as regards age, smoking index and socioeconomic status (age range 24 - 55, mean 38.6±10.26 years).

The small factory was involved in the production of wooden furniture and metal furniture frames, metal shelves and shelf supports, coat-hangers. The factory comprised separate workshops including, carpenters workshop, furniture fabrics and coverings, furniture painting, and dry powder coatings. The workshops were not well ventilated, with one exhaust ventilator and a ceiling fan in each one.

Solvent based paints are used in the furniture painting workshop. Different types of pigments, resins, and solvents are used in its manufacture.The main solvents used are toluene, xylene together with paint thinners.

Powder coating is the technique of applying dry paint to a part. Powder coating is typically produced by blending and extruding together resins, curing agents, pigments and additives. The resultant matrix is ground into fine discrete particles. Such powders are applied to a substrate or work piece via a pressurized spray application system, complete with electrostatic charging of the powder coating to charge the particles and affect a high level of transfer on to the work piece. Application can be via either fully automated or manual systems.In our study the application was all manual. The work-piece is transported through a spray zone and the powder is applied via spray guns and the part is then placed in an oven and the powder particles melt and coalesce to form a continuous film. During the curing process (in the oven) a chemical cross-linking reaction is triggered at the curing temperature and it is this chemical reaction which gives the powder coating many of its desirable properties.

Methods:

All subjects underwent:

1-An interview based on a previously prepared questionnaire including: Full occupational and medical history emphasizing on the possible clinical manifestations from exposure to solvents and dry painting: Skin, eye and respiratory tract irritation and allergic manifestations, CNS, CVS effects, reproductive history, any history of neoplasms (benign or malignant)

2-Laboratory investigations: A first morning urine sample from the day of the examination was collected and stored in a urine container (25ml). Blood specimens were obtained from the cubital vein. Heparinized whole-blood samples (5ml) for cytogenetic analysis

* Urine Malondialdehyde,Peroxinitrile, and Total Sulphydril groups level. These are indicators of oxidative stress

Urine MDA was assayed using the standard technique described by (Stringer et al., 1989).

Determination of total sulfhydryl groups, protein-bound sulfhydryl groups, and freesulfhydryl groups in urine samples using DTNB (Ellman's reagent) (Sedlak and Lindsay, 1968 and Bulajetal., 1998).

Determination of peroxynitrile in urine samples was done using the technique described by (De-Jia et al., 2004)

***Cytogenetic analysis**

The collected blood samples were kept in an upright position for two hours then cultured on a culture medium (RPMI 1640 gibco)+phytohaemoglutinin supplemented with L-glutamine and fetal calf serum. Cultures were incubated at 37 °c for 72 hours. Colchicine was added 30 minutes prior to harvest at a final concentration of 0.1 mg/ml. The cultures were centrifuged for 10 minutes and the supernatant fluid was discarded. Hypotonic solution was added to cultures and kept for 30 minutes at 37ºC . Then the cultures were centrifuged for 10 minutes and the supernatant fluid was discarded . The cultured cells then were washed by addition of a fixative solution (methanol :glacial acetic 3:1). The process of fixing cells was repeated for 3-4 times . After the last time of washing , part of the supernatant fluid was discarded and the rest was mixed with the ppt. 4-6 drops were allowed to drop on a cold wet slide and left to dry . The slides were stained for 5 minutes in 10%Gimsa stain in pH 6.8 (Verma&Babu,1989) .

For analysis, 30 well-spread chosen metaphases were counted and scored for chromosome changes by direct observation at X 1,250. Observed aberrant cells were recorded and scored for morphological and numerical aberrations. The chromosomal aberrations were classified as structural aberrations such as chromatid gap (G'),isochromatid gap (G"), chromatid break (B'),isochromatid break (B"),dicentric(Dic), separation (Sep) and deletion (Del) and numerical aberrations.

Statistical analysis:

Results were evaluated for each group. Data were compared using different tests according to the type of data. The unpaired student "t test" was used for comparing the means of both groups. The chi2 test was used to perform qualitative comparison between the different groups. The statistical significance was defined as P-value <0.05. Analysis of variance (ANOVA) was used for multiple comparisons between the groups. Pearson correlation test was used to correlate between different variables among the exposed group. The statistical significance was defined as P-value <0.05. Computer based statistical package for social sciences (SPSS) for windows 16 program was used.

Results:

The study population consisted of 65 males divided into 34 exposed and 31 control groups.The exposed group was comprised of all workers exposed to paint in the painting unit of a small scale factory in Cairo involved in furniture production and painting.The control group is composed of 31 workers involved in administrative and office work and never worked in paints and not exposed to genotoxic chemicals, the control subjects were chosen so that to match the exposed group as regards age, smoking index and socioeconomic status.

The exposed workers were further subdivided into two subgroups, group 1 (n=23) comprised workers occupationally exposed to solvent based paints, and group 2 (n=11) comprised workers exposed to dry powder coating type of painting. No significant difference between both groups as regards age, SI or duration of work.

The exposed workers age ranged (24-59years) with a mean 41.91±10.64 years with no statistically significant difference with the control group (age range 24 - 55, mean 38.6±10.26 years).

Smoking was more prevalent in the control group (SI 19.5± 17.96) but with no significant difference when compared to the exposed group (SI 16.34 ±19.76).

As for the duration of work of the exposed workers mean 20.89±10.15 years.

As regards the symptoms among the study population, data not presented, the main complaint was in the form of skin, eye and upper respiratory tract irritation, and three exposed subjects were under treatment from bronchial asthma, there was no statistically significant difference between the exposed and control groups as regards clinical manifestations.

Evaluating the level of chromosomal aberrations in the exposed and control groups revealed structural changes but no numerical abnormalities. The structural aberrations in the form of break /gaps, isochromatid breaks/ isochromatid gaps, and deletions were all found to be more than doubled among the exposed group in comparison to the control group. The difference was found to be highly significant. As for the dicentric and separation, the mean level of the aberrations was higher among the exposed than control and the difference was statistically significant. No significant difference between both groups as regards level of fragments.

Regarding the oxidative stress indicators, the mean level of MDA, Peroxynitrile was statistically significantly higher and the total sulphhydril groups were significantly lower in the exposed workers compared to the control.

Further comparison of the exposed subgroups and control group was done using Analysis Of Variance test (ANOVA). Evaluation of the level of chromosomal aberrations and oxidative stress indicators, revealed significant difference regarding all parameters between various groups.

Post Hoc test (table 4) showed that the mean level of all aberrations except separation was highest in group 1 (solvent based painters) compared to group 2 (powder dry painters) and control group, and the difference was highly significant between group 1 and group 2 for all aberrations.

Mean level of aberrations was higher in group 2 compared to the control group, but the difference was not significant for all except for break/ gap type aberrations.

Regarding the oxidative stress indicators, mean level of MDA and PN was highest in group 1compared to group2 and the control group, and the level was lowest for SHG.

Post Hoc test showed that the difference in the mean level of MDA was statistically significant between group 1 and group 2, and the difference was non significant for PN and SHG.

On the other hand the mean level of MDA and PN was higher and the total SHG was lower in group 2 compared to the control and the difference was statistically significant.

Correlation between different variables (data not presented) revealed non significant positive correlation between chromosomal aberrations with duration of work, age and smoking index. Regarding oxidative stress indicators all correlations were not significant; the correlation was positive for MDA and PN and negative for SHG with duration of work, age and smoking index.

The correlation between oxidative stress (higher MDA and PN, and lower total SHG) and chromosomal aberrations was positive (not significant correlation).

Table (1): Mean ± SD of age, smoking index and duration of work of the studied population.

	Cases (n=34)	**Control (n=31)**	**t-test**	**P**
Age	41.91±10.64	38.6±10.26	1.26	>0.05
SI	16.34±19.76	19.5±17.96	0.06	>0.05
Duration of work	20.89±10.15			

Table (2) Mean ± SD of chromosomal aberrations and oxidative stress indicators in the exposed and control groups.

	Cases (N=34)	Control (N=31)	t	P
Break/gap	10.32± 4.6	2.3 ± 1.57	9.06	*< 0.0001
Isobreak/Isogap	6.79 ± 4.8	1.2 ±0.76	6.2	*< 0.001
Deletion	1.47 ± 1.89	0.5± 0.68	2.65	*<0.05
Fragments	0.9 ± 1.3	0.5 ± 0.68	1.66	>0.05
Dicentric	0.21± 0.53	0.0 ±0.0	2.09	*<0.05
Separation	0.41± 0.6	0.1±0.30	2.53	*<0.05
MDA	0.071±0.063	0.013±0.014	4.906	*<0.001
PN	1.22±13.2	1.004±61.5	8.105	*<0.001
SH	0.102±0.086	0.220±0.1629	3.689	*<0.001

MDA= Malondialdehyde (mmol/ gmcreatinine)
PN= Peroxinitryle (nmol/ mg creatinine)SH= Total Sulphhydril Groups (mmol/ mg protein)
* Significant P value

Table (3): Analysis Of Variance (ANOVA) test of mean ± of chromosomal aberrations and oxidative stress indicators in the exposed subgroups and the control groups.

	Group 1 (N=23)	Group 2 (N=11)	Control (N=31)	F	P
Break/gap	12.09±4.42	6.64±2.24	2.3 ±1.57	68.8	*<0.001
Isobreak/Isogap	8.65 ± 4.67	2.91 ± 2.47	1.2 ±0.76	40.6	*<0.001
Deletion	1.91 ± 2.13	0.54 ±0.687	0.5 ± 0.68	7.4	*<0.001
Fragments	1.34± 1.4	0.09± 0.301	0.5 ± 0.68	7.86	*<0.001
Dicentric	0.30 ± 0.63	0.1 ± 0.0	0.0	4.69	*<0.05
Separation	0.217 ± 0.42	0.818 ± 0.75	0.1 ± 3.5	10.54	*<0.001
MDA	0.0926±0.0654	0.0284± 0.0282	0.0137±0.01427	23.8	*<0.001
PN	1.224± 182.9	1.2200 ± 116.885	1.0047± 61.584	32.69	*<0.001
Total SHG	0.0645± 0.08606	0.120217±0.0830	0.2206 ± 0.1629	7.55	*<0.001

* Significant P value

Table (4) : Multiple comparisons (Post Hoc Test) of the mean difference in chromosomal aberrations and oxidative stress indicators in the various groups:

			Mean Difference	Std. Error	significance	95% Confidence Interval	
						Lower Bound	Upper Bound
Break/ Gaps	1	2	5.45059*	1.10344	.000	3.2441	7.6571
	1	C	9.78696*	.83422	.000	8.1188	11.4551
	2	C	4.33636*	1.06097	.000	2.2148	6.4579
Isobreak/ Isogap	1	2	5.74308*	1.10966	.000	3.5242	7.9620
	1	C	7.45217*	.83893	.000	5.7746	9.1297
	2	C	1.70909	1.06696	n.s	-.4244	3.8426
Deletion	1	2	1.36759*	.50998	.009	.3478	2.3874
	1	C	1.41304*	.38556	.001	.6421	2.1840
	2	C	.04545	.49035	n.s	-.9351	1.0260
fragments	1	2	1.25692*	.35631	.001	.5444	1.9694
	1	C	.84783*	.26938	.003	.3092	1.3865
	2	C	-.40909	.34260	n.s	-1.0942	.2760
dicentric	1	2	.30435*	.13979	.033	.0248	.5839
	1	C	.30435*	.10568	.005	.0930	.5157
	2	C	-.30435	.13979	n.s	-.5839	-.0248
separation	1	2	-.60079*	.16428	.001	-.9293	-.2723
	1	C	.11739	.12420	n.s	-.1310	.3657
	2	C	.71818	.15795	n.s	.4023	1.0340
MDA	1	2	.0641542*	.0154402	.000	.033280	.095029
	1	C	.0789087*	.0116731	.000	.055567	.102251
	2	C	.0147545	.0148460	.004	-.014932	.044441
PN	1	2	-24.4596838	4.0512121E1	n.s	-105.468677	56.549309
	1	C	215.2930435*	3.0628038E1	.000	154.048497	276.537590
	2	C	239.7527273*	3.8952985E1	.000	161.861420	317.644035
SH	1	2	.0556719	.0468443	n.s	-.037999	.149343
	1	C	-.1004493	.0354153	n.s	-.171267	-.029632
	2	C	-.1561212*	.0450415	.001	-.246187	-.066055

*. The mean difference is significant at the 0.05 level.

1= solvent based painters group , 2= powder paint group, C= control group

Discussion:

Paint is a generic name for a number of different products, and its potential toxicity depends on the types of pigments, resins, and solvents used in its manufacture. One of the major groups of paints is latex paints, for which the resin is acrylic-, vinyl-, or styrene-based and the solvent is water, with the customary addition of glycol ethers and coalescent aid that helps the resins flow together, aiding in film formation. The other is the alkyd paints or solvent based paints, in which the solvent is usually petroleum-based and organic, such as toluene or xylene.The third group is Industrial thermosetting powder coating (dry paints). This is typically produced by blending and extruding together resins, curing agents, pigments and additives. The resultant matrix is ground into fine discrete particles (Kirk-Othmerm, 2006).

Powder coatings are applied to a substrate or work piece via a pressurized spray application system, complete with electrostatic charging of the powder coating to charge the particles and effect a high level of transfer on to the work piece. Application can be via either fully automated or manual systems, with the work-piece transported through a spray zone containing a number of guns and into a stoving oven via an overhead conveyor. Air pressures and electrical potentials are employed. Systems are designed to minimize the amount of overspray. Excess powder is removed by exhaust extraction and collected for re-use or disposal (CEPE, 2005).

The health effects of organic solvents have been known for more than a century, as described by Winslow (1927). At low or moderate concentrations, the organic solvents may cause transient symptoms such as euphoria, headache and dizziness. At high concentrations, anaesthesia and disturbances in respiration and circulation may occur and may lead to death. These effects are known from occupational exposure and from organic solvent abuse. In addition to these acute effects, organic solvents are known to cause adverse effects in the nervous system after long-term exposure (Mandiracioglu et al., 2011). WHO accepted the occurrence of a chronic organic solvent intoxication syndrome in 1985, and Juntunen (1986) described the syndrome thoroughly. The syndrome typically develops gradually after exposure

to organic solvents daily for many years. The syndrome may occur in several occupations where the workers are exposed to organic solvents.

Solvent based paints, for generations, were the only available surface coating for industrial use on surfaces. Chemistry and creativity, however, have yielded several other options in recent years. First among these is a dry-application known as (Powder Coat).

Powder coatings are a combination of resins, pigments, fillers, flow control agents, and catalysts. While some of these ingredients are considered toxic, studies confirm that most are relatively inert and safe when tested in formulated powder because of absence of solvents. For this reason, most powder coatings are classified nuisance dust instead of hazardous. Inhalation and skin contact are the likely means of exposure to powder dust (White, 2004)

Evaluating the level of chromosomal aberrations in the exposed and control groups revealed structural changes but no numerical abnormalities. The structural aberrations in the form of break /gaps, isochromatid breaks/ isochromatid gaps, and deletions were all found to be more than doubled among the exposed group in comparison to the control group, the difference was found to be highly significant. No significant difference between both groups as regards level of fragments.

In our study we attempted also to assess the oxidative stress status in the studied population, as indicators of oxidants two parameters were investigated the product of lipid peroxidation MDA, and peroxinitrile, and total sulphhydril groups as indicator of antioxidant status. Regarding the oxidative stress indicators, the mean level of MDA and Peroxynitrile was significantly higher and the total sulphhydril groups were significantly lower in the exposed workers compared to the control, indicating an oxidative stress status in the exposed workers.

Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as a disturbance in the equilibrium status of pro-oxidant/ anti-oxidant systems in intact cells. This definition of oxidative stress implies that

cells have intact pro-oxidant/anti-oxidant systems that continuously generate and detoxify oxidants during normal aerobic metabolism. When additional oxidative events occur, the pro-oxidant systems outbalance the anti-oxidant, potentially producing oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, ultimately leading to cell death in severe oxidative stress. Mild, chronic oxidative stress may alter the anti-oxidant systems by inducing or repressing proteins that participate in these systems, and by depleting cellular stores of anti-oxidant materials such as glutathione and vitamin E (Scélo et al., 2009).

Recent work demonstrates the formation of peroxynitrite, and its potential pathogenetic relevance in cells or animals exposed to various environmental toxins (Szabo, 2003)

Simultaneous generation of nitric oxide (NO) and superoxide favors the production of this toxic reaction product, peroxynitrite anion (ONOO_). Peroxynitrite and its conjugate acid are strong oxidants, and are particularly effective oxidant of aromatic molecules and organosulfur compounds that include free amino acids and peptide residues. Peroxynitrite reported activities include a rapid oxidation of sulfhydryl groups and thioethers, as well as nitration and hydroxylation of aromatic compounds, including tyrosine, tryptophan and guanine (Greenacre and Ischiropoulos, 2001).

The various reactions of peroxynitrite with enzymes, macromolecules and lipids, have been shown to influence cellular functions. Peroxynitrite enhances and triggers a variety of pro-inflammatory processes. For example, peroxynitrite enhances the expression of ICAM-1 and P-selectin in human endothelial cells and it mediates the cytokine-induced IL- 8 expression in human leukocytes. In human neutrophils, peroxynitrite triggers the down-regulation of L-selectin expression, and up-regulation of CD11b/CD18 expression (Zouki et al., 2001).

The reaction of peroxynitrite with lipids leads to peroxidation (malondialdehyde and conjugated diene formation) and formation of nitrio-, nitro-, nitrosoperoxo- and/or nitrated lipid oxidation adducts. It is important to note that peroxynitrite can inhibit superoxide dismutase glutaredoxin

and other antioxidant molecules and systems. Peroxynitrite-mediated depletion of one of the key cellular antioxidants, glutathione can lead to positive feedback cycles of intracellular oxidant generation and exacerbation of the oxidative cellular injury (Aykac-Toker et al., 2001).

An important interaction of peroxynitrite occurs with nucleic acids, Peroxynitrite-induced DNA single strand breakage. Peroxynitrite is more cytotoxic than NO or superoxide in a variety of experimental systems. In fact, recent studies suggest that, peroxynitrite, and not NO, may be the ultimately cytotoxic species in many conditions. In cells exposed to authentic peroxynitrite or to compounds that simultaneously generate NO and superoxide, marked changes in the level of cellular energetic and DNA integrity occur (Virag and Szabo , 2002).

The products of lipid peroxidation are easily detected in blood plasma and have been used as a measure of oxidative stress. The most commonly measured product is malondialdehyde

Malondialdehyde (MDA) is an endogenous genotoxic product of enzymatic and oxygen radical-induced lipid peroxidation. MDA is a potentially important contributor to DNA damage and mutation. MDA is mutagenic in bacterial and mammalian cell assays, and it is carcinogenic in rats (Laura et al.,2003.)

The maximum increase in mutation frequency observed after MDA treatment was 15-fold above background levels. This level of increase is comparable with the mutation frequencies induced by UV light and by another endogenous genotoxin, nitric oxide (Routledgeet al.,1993).

In organic chemistry, a thiol is anorganosulfur compound that contains a carbon-bonded sulfhydryl (-C-SH or R-SH) group (where R represents an alkane, alkene, or other carbon-containing moiety). Thiols are the sulfur analogue of alcohols, and the word is a portmanteau of “thio” + “alcohol,” with the first word deriving from Greekθειον (“thion”) = “sulfur”. The -SH functional group itself is referred to as either a thiol group or a sulfhydryl group.The low molecular weight thiol, glutathione, and “reactive” protein sulfhydryls (exposed cysteines in many proteins) are primary participants in

cellular anti-oxidant systems. Glutathione is abundant in cytoplasm, nuclei, and mitochondria and is the major soluble anti-oxidant in these cell compartments. Reactive protein sulfhydryls are abundant in both soluble proteins and in membrane-bound proteins. Important antioxidant in the detoxification of xenobiotics, carcinogens, free radicals and maintenance of immune functions as a nonproteinthiol. By this way, considerable evidence points to the fact that sulphydryl compounds play an important role in the cellular response to xenobiotics(Unsal et al, 2005).

Numerous recent evidence supports the role of oxidative stress in the pathophysiology of Genotoxicity induced by the exposure to solvent based paints (KaragÖzler et al, 2002, Khan et al., 2010 and Moro et al., 2010).Exposure to chemicals in paints can induce an oxidative stress via the depletion of glutathione (GSH) and other antioxidant defences and that this may lead to indirect genotoxicity(Beddowes et al.,2003 , Sardas et al.,2010)

Oxidative stress occurs through the generation of reactive oxygen species and by reducing the antioxidant cell defense systems by depleting glutathione, by inhibiting sulfhydryl-dependent enzymes, by interfering with some essential metals needed for antioxidant enzyme activities, and/or by increasing cell susceptibility to oxidative attack by altering the membrane integrity and fatty acid composition (Moro et al., 2010). Consequently, the resulting impaired oxidant/antioxidant balance can be partially responsible for the effects. Exposure to chemicals in paints can induce an oxidative stress via the depletion of glutathione (GSH) and other antioxidant defences and that this may lead to indirect genotoxicity(Beddowes et al.,2003 , Sardas et al.,2010)

Similar results were found by Patil et al., 2007 who assessed the biochemical, hematological and antioxidant status of a group of spray painters of Western Maharashtra (India). The serum malondialdehyde (MDA) content was significantly increased ($p<0.001$) and the activities of antioxidant enzymes were significantly reduced in the exposed group as compared to control group. The study clearly indicated an imbalance of prooxidant/ antioxidant status in spray painters

Decreased antioxidants activities in painters may indicate a possible role of lead-induced generation of O2.- and H2O2. Thus it is speculated that Pb2+ may induce generation of reactive oxygen species by interacting with oxy-haemoglobin, which may lead to per-oxidative damage of RBC membrane. This theory accumulated is reported in several studies (Monteriro et al., 1991; Hermes Lima et al., 1991). Heavy-metal-induced alteration of antioxidant enzyme activities and nucleic acid concentration in painters were also reported in other studies (Das and Das, 2004). Similar results were found by (Długosz et al., 2005), oxidative stress indicators were significantly higher in painters, and they deduced that it was not attributed to a single solvent exposure in the paint but rather the synergistic effect of multiple chemicals exposure in the paint material that results in oxidative stress. Moro et al., 2010 analyzed the levels of biomarkers of exposure for toluene, xylene, styrene, ethylbenzene, and lead, as well as the oxidative stress biomarker alterations in solvent based painters. The results demonstrated that despite the fact that all the biomarkers of exposure were below the biological exposure limits, the MDA levels and antioxidant enzyme activities were increased, while nonproteinthyol groups were decreased in painters when compared with nonexposed subjects.

Genotoxicity induced in painters was demonstrated in several studies, in agreement with our study. Pinto et al.,2000 results clearly demonstrated genotoxicity among the painters population as evident from increase micronucleus frequencies, frequent nuclear changes, and apoptosis . Cytogenetic damage was significantly associated with occupational exposure time. Madhavi et al., 2008 and Sardas et al.,2010 found a significant increase in the frequency of chromosomal aberrations in the workers when compared to the controls. Further, smoking had an added effect on the frequency of aberrant metaphases

Siebel and Da Silva, 2010 found that painters working in auto body shops were at risk for genotoxic damage, while office workers seem to be protected. They also concluded that age, duration of work, use of protective masks, alcohol consumption, and smoking habit were not significantly correlated to micronuclei level. In our

study the chromosomal aberrations were positively correlated with age, duration of work and smoking index but the correlation was not significant.

Compared to our findings, (Silva et al., 1996) found a significantly higher frequency of aneuplodies and chromosome deletions in the peripheral lymphocytes of car painters than in control subjects. They also detected a significant correlation between the time worked as a car painter and the frequency of aneuploidy. Smoking habits do not represent a significant factor in terms of production of the various types of chromosome aberrations among car painters. Gajalakshmi 2002 found baseline frequency of chromosomal aberrations was significantly higher among painters as compared to matched controls. Smoking and alcoholism as modulating factors had no added effect on the frequency of aberrant metaphases. Stepwise multiple linear regression analysis indicated that duration of service and age were significant factors that influence the frequency of chromosomal aberrations observed.

Evaluation of the level of chromosomal aberrations and oxidative stress indicators, in the two subgroups of the exposed workers and the control group, the results revealed significant difference regarding all parameters between various groups (table 3).

Our results showed that the mean level of all aberrations except separation was highest in group 1 (solvent based painters) compared to powder dry painters and control group, and the difference was highly significant between group 1 and group 2 for all aberrations except for break/ gap type aberrations.

The mean level of all aberrations was higher in group 2 (dry painters) except for fragments compared to the control group, but the difference was not significant.

The oxidative stress was most evident in the solvent based painters compared to the dry painters and the control and the difference was significant for all parameters when compared to the control.

The above results indicate that oxidative stress and genotoxicity were evident in the solvent based painters more than the dry painters, but on the other hand dry painting was not completely safe, due to the higher level of oxidative stress indicators and

chromosomal aberrations (although not significant except for peroxinitrile and total sulphhydril groups) in this group compared to the control.

Many studies have deduced the genotoxicity effect of occupational exposures in solvent based painters, and the possible role of oxidative stress was investigated(Karagözler et al, 2002, Khan et al., 2010 and Moro etal., 2010).

Further studies are needed to fully characterize the adverse health effects in workers occupationally exposed to dry powder coatings, however the idea that the powder coating is simply exposure to nuisance or irritant substances is definitely changing and in our study the significant difference between group 1 and group 2, and the difference although not significant between group 2 and the control group, indeed proves that.

Conclusion and recommendations:

This study showed that workers exposed to paints have a significant increased level of chromosomal aberrations and indicators of oxidative stress. This is in accordance with other studies that claim the role of oxidative stress in the pathophysiology of genotoxicity in painters especially those exposed to solvent based paints, in which the solvent exposure carries an additional risk to adverse effects.

Our findings revealed a lower level of hazard when using powder coatings compared to conventional solvent-based paints based on the fact that the findings were more evident in the workers exposed to solvent based paints compared to workers involved in dry powder coatings.

However, the higher level in the chromosonmal aberrations and oxidative stress indicators in the dry powder coating exposed workers compared to the control, indeed raises suspicion about the safety of powder coatings as a "Safer Alternative to Solvent Based Paints". Indeed, further larger scale studies are needed for more clarification of this issue.

Solvent based paints are indispensable, and their alternative (powder coating) application is increasing. Adopting safe working practices will definitely reduce the risks to a minimum. Engineering controls and regular health surveillance of the workers should be adequately applied. Personal protective equipments should be

provided (anti-static coveralls designed to prevent ingress of the powder, suitable gloves to minimize skin contact and goggles to protect the eyes). There is also an intense need for better training of workers involved in handling and application of paints.

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