ADVERSE HEALTH EFFECTS ASSOCIATED WITH INCREASED CYTOGENETIC DAMAGE AND *ERCC2* RISK GENOTYPE IN THE OCCUPATIONALLY EXPOSED WASTE HAIR RE-CYCLING WORKERS FROM WEST BENGAL, INDIA

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Abstract: Waste poses environmental problem both nationally and globally when its transformation into resource becomes a challenge. Literature evidences show utilization of waste hair in various industries; also on the contrary showed negative impact on hairdressers. The present study aims to identify the adverse health impacts, cytogenetic damage and genetic susceptibility among waste hair reprocessing workers.Total 1002 study participants including 502 workers and 500 healthy individuals were recruited for the epidemiological survey from East Midnapore district of West Bengal. Out of them, 213 individuals (109 worker, 104 non-workers) gave their consent to provide biological samples (blood, urine and sputum) to carry on molecular cytogenetic analyses.The epidemiological survey showed prevalence of respiratory problems (OR 4.089; 95% CI, 2.57-6.49, p<0.001), allergic manifestations (OR 21.12; 95% CI, 9.17-48.62, P<0.001) and musculoskeletal pain (OR 46.34; 95% CI, 16.99-126.40, p<0.001) were significantly higher among workers compared to non-workers. Urine sample analysis showed higher urothelial micronuclei (3.87/ 1000 cells vs. 1.02/ 1000 cells, p<0.005) while sputum sample analysis revealed increased alveolar macrophage (176 cells/ 15 hpf vs. 48 cells/ 15 hpf, p<0.005) with elevated nuclear anomalies in workers compared to non-worker. Codon 751 Lys/Lys variant in *ERCC2*, a Nucleotide Excision Repair (NER) gene was studied and found, workers had higher association with Lys/Lys (63.3% vs. 30.7%, OR 4.24, 95% CI=2.35-7.65 p =<0.0001) compared to non- workers. Increased awareness amongst the workers with systematic but effective utilization of waste will significantly improve socio-economic conditions benefitting both worker and society.

Keywords: Waste hair; occupational risk; adverse health effect; cytogenetic damage; individual susceptibility

I. INTRODUCTION

Human waste hair is considered as a bio-waste throughout the world. Slow natural decomposition and improper disposal create a multitude of environmental problems including blocking of sewage system, water pollution, eutrophication as well as health hazards like respiratory troubles, allergic manifestation, musculoskeletal pain and co-morbidities (Brebu et al., 2011; Gupta, 2014; Hassan and Bayomy, 2015). Interestingly, some properties of human hair, especially of Indian origin, are very unique for its chemical composition, slow degradation rate, high tensile strength, thermal insulation, elastic recovery, scaly surface and interaction with water and oil (Gupta, 2014). These properties could be efficiently utilized for versatile application in agricultural industries as fertilizers (Zheljazkov et al., 2008; Oh et al., 2011), as bio-adsorbents in removing dyes and heavy metals from pollutant water (Banat et al., 2001; Malliga et al., 2010), in pharmaceuticals and biomedical industries as a rich

source of amino acids and most importantly in fashion industry for wig making, hair extension etc (Gupta, 2014). Although wigs are widely available in markets, little is known, how the wigs are made and the health risk of the people associated in the process of wig making. Related literatures are also not readily available regarding safe processing of waste hair. The aim of the present article is to allow a better understanding of work flow for wig processing and consequent health impacts on the workers, who are unaware of safety guidelines. The varied applications of processed hair make this prolific industry viable. To meet the demand from downstream industries waste hair reprocessing is also growing synergistically. Despite huge economic turnover (Gupta, 2014), this occupation remains as a cottage industry, popular in the semi urban and rural areas of India and awareness of the workers regarding environment, health and safety are limited. The workers are exposed to various chemicals including potential carcinogens that primarily enter into the body, either through dermal exposure or inhalation. Moreover, workers are frequently exposed to detergents (soaps and shampoos), bleaching agents containing per-sulphate salts, hair dyes containing Paraphenylene diamine (P-pD) and Hydrogen peroxide (H_2O_2). Although the products are often labelled as herbal or safe, they contain various ingredients, often lead or other metals which can be graded mild to moderate allergens (Cohen and Roe, 1991; Goebel et al., 2018; Hamann et al., 2018; Han et al., 2018) that cause various skin diseases including irritation, rashes and contact dermatitis. Some of these chemicals are released in gaseous state as well as in particulate form along with a number of surface contaminants which remain air borne in the work environment at the breathing zone. Long term inhalational exposure of such chemicals which act as airway irritants (Parra et al., 1992; Sala et al., 1996) may cause increased risk of asthma and other respiratory ailments. A number of epidemiological studies identified the increased risk of skin, aero-digestive tract, lung, colorectal and bladder cancer among the hair dressers (Czene et al., 2003; Takkouche et al., 2009). While review of literature is available on biology of human hair and its growth, socio-cultural aspect and adverse health impact on hair dressers; no research has so far being conducted on hair processing workers to understand the negative impact on health integrating epidemiology, cytogenetic and genetic variant analysis.The present paper explores the health risk associated with the waste hair processing workers (more than 10 years at job), examines the cytogenetic damage and at the same time assesses the individual genetic susceptibility. To reduce the occupational health risk of the associated workers, there is a great need for awareness in the workers and develop efficient and at the same time environmentally safe waste management system.

II. Materials and methods

2.1. Recruitment of study participants

A cross sectional study was conducted from May 2015 through June 2018. Initially a total of 2100 individuals were interacted for this study, of which 1500 people were associated with waste hair reprocessing and 1000 people were from non-waste hair reprocessing occupations. All the participants were from the same area of Radhapur village in Purba Midnapore district of West Bengal. Finally 502 individuals from waste hair reprocessing category were included in the epidemiological survey as they were full time workers (6-8 hours/day) for more than 10 years and were interested in the long term follow up of the study. Part time workers (<7 hours/day) and individuals associated with this occupation for less than 10 years were excluded for the present study. Detailed work flow of study participant recruitment and sample collection was elaborated in Fig 1. The study was approved by the ethical review committee of University of Calcutta (0010/16-17/1356 dt 5/10/2016).

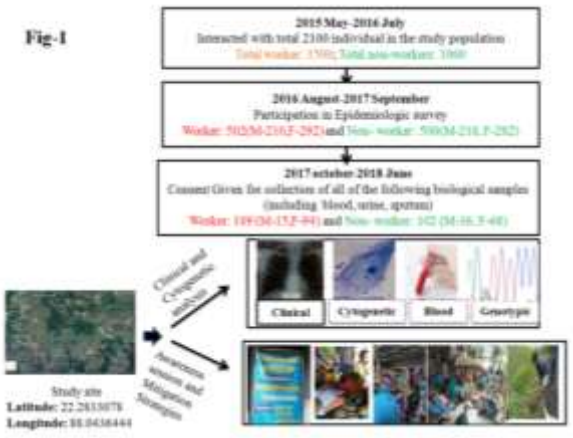

Fig 1. Schematic representation of study participant selection and work strategy.

2.2. Study of urothelial micronuclei from urine sample

Quantitative analysis of micro nucleus (MN) in the urothelial cells was measured following the method previously described by Ghosh et al., (2006). Urine samples were collected in the sterilized container (Sterile Uricol, Himedia) from study participants. Urinary epithelial cells were collected by centrifugation (1,000 rpm for 10 min). Cell pellets were washed with 0.9% NaCl (Merck, India) and cell density was observed with a phase contrast microscope. Cell solution was either concentrated by centrifugation or diluted in 0.9% NaCl, as per requirement. After getting the desirable cell density ($1.5–2.0 \times 10^6$), 50 µl of the cell suspension was spread on clean and preheated glass slides and allowed to air dry for 10 minutes. The

slides were then treated with fixative (methanol: acetic acid = 3:1, Merck, India) and kept for few minutes (10-12m) in room temperature for air dry. Finally stained with Giemsa (Merck, India), and observed under the microscope for analysis. At least, 1000 cells from each individual were analysed and the cells having intact nucleus were considered (clumped and necrotic cells were excluded). Scoring of MN was done by two trained research fellows and noted only with >80% concurrence. MN with the following criteria were selected - distinctly separated from the main nucleus, in the same focal plane as the nucleus, of the same color, texture and refraction as the main nucleus which were and less than 1/3 of the diameter of the main nucleus.

2.3. Study of Alveolar macrophage from Sputum sample

Papanicolaou staining of sputum samples were done to observe the presence of alveolar macrophage (AM), following the previously reported method of Hughes and Dodds (Lahiri et al., 2000). Sputum samples were collected on sterilized watch glasses and fixed on slides. Each slide was passed through graded alcohol and then stained with Harris' hematoxylin (Merck, India) for 2 minutes. It was then washed in running tap water and dehydrated in 95% alcohol. Finally, the slides were counter-stained in orange-G (Merck, India) for 2 minutes, washed in 95% alcohol followed by staining in EA-50(Merck) mixture for 2 minutes and dehydration in absolute ethanol. The slides were dipped in xylene and mounted in DPX prior to microscopic study. Frequency of AM was scored from 15 high power fields (hpf) for each slide. Only the intact AM cell was recorded and the clumped, overlapped or smeared cells were excluded from individual scoring. The scoring procedure and the selection criteria for MN were same as described in the previous section (2.2).

2.4 Analysis of genetic variant

About 5 ml of blood sample was collected from each study participant and genomic DNA was extracted from whole blood using QIAamp DNA Mini Kit (QIAGEN, GmbH, Germany). Polymerase chain reaction (PCR) was done in 20 µl reaction volume using GoTaq Green master mix (Promega) in a thermal cycler. Primers and PCR condition was taken from previous study by Banerjee et al., 2007. PCR products were used for restriction digestion with EarI (New England Biolabs Inc. Beverly, MA) in a 20 µl digestion mixture at 37ºC for 4 h. EarI restricted products of codon 751 Lys/Lys genotypes (AA) have band sizes of 262 and188 bp, while a band of 450 bp corresponds to Gln/Gln genotype (CC) with a loss of EarI restriction site for 'C' allele. Therefore, heterozygote Lys/Gln genotype (AC) have all the three bands of sizes 450, 262 and 188 bp. Digested products were electrophoresed in 2% agarose gel, stained with ethidium bromide and visualized under UV gel imager. Genotyping of 30% of the samples were reconfirmed by DNA sequencing.

2.4 Statistical analysis

Statistical analyses were done using minitab software version 18. To validate the difference in AM, BN, MN and UMN level between exposed and control groups, two sample t-test (unequal variance) was used. Further, to assess the influence of confounding factors, we used ANOVA by general linear model (GLM), taking AM, BN, MN, UMN as response and the exposure status along with confounding factors as explanatory variables and age as covariate. To explore the difference between exposed and control group in terms of multiple responses (AM, BN, MN and UMN together), we had also constructed a multivariate GLM taking the afore-mentioned combination of explanatory and covariate variables.

III. RESULT

3.1. Demographic Data

The epidemiological study was conducted on 502 workers and 500 non-workers. Detailed demographic description of the study population has been summarized in Table 1. Majority of the workers are females, i.e. 58.17% and none of them are addicted to any kind of tobacco products. About 41.83% of study population were male and all of them were addicted to tobacco habits (either chewing or smoking). The workers were associated with this occupation for more than ten years with an average working duration of seven hours per day (self-reported), indicating their prolonged occupational exposure towards hair particles (Fig 2).

Fig 2. Representative photographs showing occupational exposure of the hair processing worker to **(A)** Hair fibres and **(B)** Chemical and dyes.

TABLE 1: Demographic characteristics of the study population

Characteristics	**Workers (N=502)**	**Non-workers (N=500)**
Age (Years)	**Workers (N=502)**	**Non-workers (N=500)**
Mean (SD)	32.30(10.8)	33.19(10.3)
Gender		
Male	210(41.8%)	218(43.6%)
Female	292(58.2%)	282(56.4%)
Smoking Habit		
Total Tobacco user	101(20.1%)	129(25.8%)
Male	101(100%)	129(100%)
Female	0	0
Total Non- tobacco user	401(79.9%)	371(74.2%)
Male	109(27.2%)	89(23.9%)
Female	292(72.8%)	282(76.1%)

3.2. EpidemiologicalSurvey

An extensive questionnaire based epidemiological study was conducted which recorded participants' (502 workers and 500 non-workers) self described health problems regarding (i) respiratory symptoms, (ii) allergic manifestation and (iii) pain related problem. Waste hair workers had higher health complaints compared to the individuals from non-workers (Table 2). According to the health related complaints for respiratory symptoms, allergic manifestation and pain related problems (considering both severity and duration) made by the participants, they were grouped into two categories “high” or “low”. Like, for respiratory symptoms, patients complaining coughing for last 3 weeks, chest pain and asthma were recorded as “high’. For allergic manifestation, patients typically complaint about skin irritation, contact dermatitis, eye irritation and nasal irritation. If a patient had 3 or more symptoms mentioned above was categorized as “high”. For pain related problems, where we considered 7 symptoms like neck pain, shoulder pain, elbow pain, wrist and hand pain, upper back pain, lower back pain and leg/foot pain; we categorized under "high", if 5 or more symptoms were self reported. The same pattern was followed for interviewing with non-worker population. Thus, for each of the 3 health related problems, we had “high” and “low” category for worker and non-worker group. The odds ratio calculation showed, workers had respiratory problem 4 times higher (OR =4.089 ; 95%CI: 2.57-6.49, p<0.001, , allergic manifestation 21 times higher (OR = 21.12; 95%CI: 9.17-48.62, p<0.001) and pain related problems 46 times higher (OR = 46.34, 95% CI: 16.99-126.40, p<0.001). Other common health problems like headache, fever, night sweat, chills and loss of appetite were also significantly higher (p-<0.001) for the workers in this occupation. Further, to address the negative consequences on workers’ health, it was indeed important to investigate the extent of cytogenetic damages in workers compared to non-workers and also to identify differential susceptibility between them, if any.

TABLE 2: Detailed description of self reported health problems of the study participants

Health Effects	**Workers N=502**	**Non-workers N=500**	***p* value**	**Odd Ratio (95% confidence interval)**	
Respiratory Problems	N (%)	N (%)			
Coughing >3 week	251(50)	52(10.4)	<0.0001**	8.61	(6.155-12.058)
Blood in cough	11(2.2)	8(1.6)	0.6467	1.37	0.549-3.454
Chest pain	93(18.6)	85(11)	0.0010*	1.839	1.284-2.635
Asthma	75(15)	25(5)	<0.0001**	3.337	2.083-5.345
The overall OR for Respiratory symptoms				**4.089**	**(95%CI= 2.57-6.49), P<0.001**
Allergic Manifestation					
Skin irritation	250(50)	88(17.6)	<0.0001**	4.64	3.478-6.201
Contact dermatitis	155(31)	68(13.6)	<0.0001**	2.83	2.064-3.900
Eye irritation	185(37)	12(2.4)	<0.0001**	23.73	13.015-43.276

Nasal irritation	171(34.2)	8(1.6)	<0.0001**	31.77	15.425-65.44
The overall OR for Allergic Manifestation				**21.12**	**(95%CI= 9.17-48.62), P<0.001**
3.2. Pain Related Problem					
Neck pain	320(64)	30(6)	<0.0001**	27.54	18.253-41.569
Shoulder pain	286(57.2)	17(3.4)	<0.0001**	37.61	22.479-62.956
Elbow pain	189(37.8)	10(2)	<0.0001**	29.58	15.421-56.768
Wrist and hand pain	209(41.8)	16(3.2)	<0.0001**	21.57	12.717-36.611
Upper back pain	148(29.6)	10(2)	<0.0001**	20.48	10.643-39.431
Lower back pain	151(30.1)	16(3.2)	<0.0001**	13.01	7.634-22.182
Leg/foot pain	158(31.6)	37(7.4)	<0.0001**	5.74	3.914-8.439
The overall OR for Pain Related Problem				**46.34**	**(95%CI= 16.99-126.40) , P<0.001.**
Other Problems					
Headache	232(46.4)	53(10.6)	<0.0001**	**7.24**	5.186-10.125
Unwanted weight lost	119(23.8)	85(17)	0.0105	**1.57**	1.111-2.070
Fatigue	174(34.8)	131(26.2)	0.0044*	**1.49**	1.139-1.959
Fever	234(46.8)	58(11.6)	<0.0001**	**6.65**	4.807-9.209
Night sweat	238(47.6)	50(10)	<0.0001**	**8.11**	5.771-11.407
Chills	119(23.8)	32(6.4)	<0.0001**	**4.54**	3.006-6.867
Appetite loss	203(40.6)	86(17.2)	<0.0001**	**3.26**	2.438-4.380

Study of urothelial micronuclei

Study of urothelial micronuclei (UMN) is a well established biomarker in toxicological assessment. Urinary epithelial cells are exfoliated in nature and prevalence of micronuclei in urothelial cells can be used as an indicator of effect biomarker. Urine sample from 109 workers and 104 non-workers were analysed, which revealed significant difference between these 2 groups (t-test, p-value < 0.001), indicating increased cytogenetic damage (Fig 3, Table 3).

Fig-3

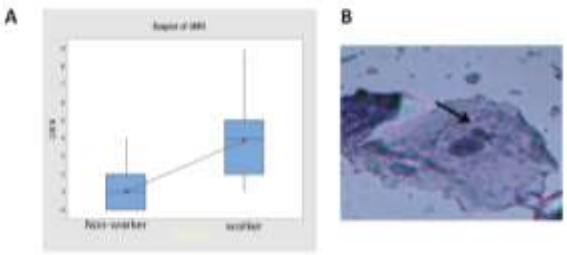

Fig 3. Study of urinary epithelial micronuclei (UMN). **(A)** Box plot representation of UMN frequency (in mean/1000 cells) between workers and non-workers. **(B)** Representative image of urinary epithelial cell with two micronuclei (arrow headed).

TABLE 3: **Comparative urothelial micronuclei frequency (per 1000 cells) between workers and non-workers**

Group	Mean	SD	p-value (2-sample t-test)
Worker (N=109)	**3.87**	**±1.92**	**<0.001**
Non-worker (N=104)	**1.02**	**±1.03**	

3.3. Study of alveolar macrophages

Presence of excess alveolar macrophages (AM) in the lower respiratory tract is an indicator of immediate allergic response due to persistent inhalation of particulate pollutants. Sputum samples were analyzed for 109 workers and 104 non-workers (Table 4). Number of AM in the sputum was expressed as the mean number of cells present in 15 high power fields (hpfs) of stained slides. Analysis of sputum samples revealed 47.8 AM/ 15 hpfs, in non-workers, while it was about 176 AM/ 15 hpfs, $p < 0.001$ in workers. Different nuclear anomalies (cells with binuclei and micronuclei) were observed in majority of AM. Two sample t-test revealed higher nuclear anomalies in the form of binucleation (11.75 AM/ 15 hpfs, $p<0.05$) or micronuclei formation (6.77 AM/ 15 hpfs, $p<0.05$) in workers compared to non-workers (Fig 4). However, such differences in AM, BN, MN and UMN levels between exposed and control groups may be influenced by several potential confounding factors like age, sex, smoking habit, individual genetic make-up etc. We constructed 4 GLM models (section 2.6) and none of them shown any confounding effect.

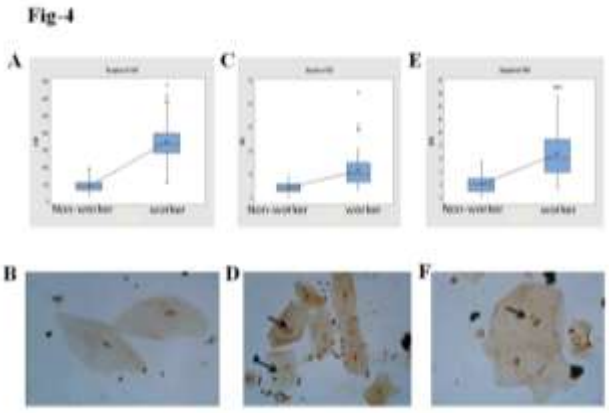

Fig 4. Study of alveolar macrophages (AM) and associated nuclear anomalies. Box plot representation with respective cellular image for total AM frequency (in mean/ 15 high power field) (A, B), Binucleated AM **(C, D)** and AM with micronuclei (E,F).

TABLE 4: Comparative analysis of total alveolar macrophage (AM) and nuclear anomalies: AM with bi-nucleation (BN) and AM with micronucleus (MN)

Group	**AM**			**BN**			**MN**		
	Mean	SD	*p*-value	Mean	SD	*p*-value	Mean	SD	*p*-value
Worker (N=109)	176.0	±52.7	<0.001	11.75	±6.77	<0.001	6.77	± 4.05	<0.001
Non-worker (N=104)	47.8	±16		4.55	±2.10		2.15	± 1.36	

3.4. Study of genetic susceptibility by ERCC2 genotyping

To investigate the individual susceptibility towards the risk of DNA damage, we selected *ERCC2* as a candidate gene (Banerjee et al., 2007) and examined codon 751 A>C polymorphism (Lysine to Glutamine) among the study participants by PCR-RFLP strategy. Individuals with Lys homozygous was 63.3% in waste hair reprocessing individuals and only 30.7% in non waste hair reprocessing group (Table 5), indicating a probable effect of *ERCC2* codon 751(Lys/Lys) genotypes with higher susceptibility towards DNA damage (OR 4.24, 95% CI=2.35-7.65, $p< 0.001$) in workers (Fig 5).There have been several attempts made to understand the interaction between occupational exposure and genetic susceptibility. Attempt has been made to identify the extent of cytogenetic damage among the study population with *ERCC2* risk genotype and it was observed with risk genotype (codon 751 Lys/Lys) in background, the occupational exposure may synergistically enhance cytotoxicity (Table 6). In addition to this, GLM model detected the difference of AM levels between worker and non-worker, which varied significantly between different genotypes of *ERCC2* (Table 7).

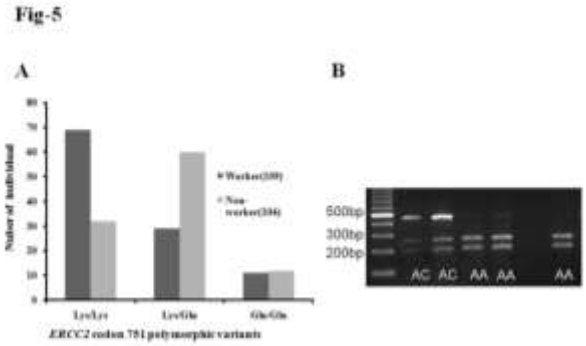

Fig 5. *ERCC2* codon 751 polymorphism. **(A)** Genetic variants in study group. **(B)** Representative gel image of RFLP digestion to detect individual genotype.

TABLE 5: Distribution of *ERCC2* risk genotype between workers and non-workers.

Group	Lys/Lys (AA) N= %	Lys/Gln(AC) + Gln/Gln (CC) N=% (AC+CC)	OR (95% confidence interval)	*p*-value
Workers (N=109)	**69(63.30)**	**34(31.19)**	**4.24 (2.35-7.65)**	**<0.0001**
Non-workers (N=104)	**32(30.76)**	**67(64.42)**		

TABLE 6 : Extent of cytogenetic damage among the study population with *ERCC2* risk genotype

Study population (Risk Genotype:Lys/Lys)	UMN	*p*-value	AM	*p*-value	BN	*p-value*	MN (AM)	*p-value*
Worker(N=69/109)	3.99	<0.001	171.82	<0.001	11.86	<0.001	6.85	<0.001
Non-worker(N=32/104)	1.125		58.81		5.15		2.15	

UMN= Urothelial Micronuclei, AM= Alveolarmacrophage,BN= Binuclei,MN=Micronuclei

TABLE 7 : **Interaction of alveolar macrophage and *ERCC2* genotype by GLM**

Alveolar macrophage(AM)					
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Age	1	1	1.2	0.00	0.977
Worker_Non-worker(1_0)	**1**	**16947**	**16946.7**	**11.91**	**0.001**
sex(Male-1,Female-2)	1	361	360.6	0.25	0.615
smoker(0-1)	1	407	407.2	0.29	0.593
ERCC2 genotype	3	4251	1417.0	1.00	0.396
age*Worker_Non-worker(1_0)	1	5131	5130.6	3.60	0.059
age*sex(M-1,F-2)	1	262	261.6	0.18	0.669
age*smoker(0-1)	1	0	0.0	0.00	0.996
age* *ERCC2*	3	1674	558.0	0.39	0.759
Worker_Non-worker(1_0)*sex(M-1,F-2)	1	18	17.7	0.01	0.911

Worker_Non-worker(1_0)*smoker(0-1)	1	4700	4699.8	3.30	0.071
Worker_Non-worker(1_0)* *ERCC2*	**3**	**23805**	**7935.1**	**5.58**	**0.001**
sex(M-1,F-2)*smoker(0-1)	1	566	565.9	0.40	0.529
sex(M-1,F-2)* *ERCC2*	3	1462	487.3	0.34	0.795

3.5. Multivariance analysis (Statistical data)

To understand the difference in frequency of all types of MN together between the exposed and the unexposed group, multivariate generalized linear model (GLM) was conducted taking multiple dependent variables (frequency of MN, BN, AM, UMN) and multiple independent variables (exposure as the effect and age, gender, genotype and tobacco habits as confounders). Considering all the confounders, it has been clearly demonstrated that elevated cytogenetic risk are strongly associated with occupational exposure ($p<0.001$).

IV. DISCUSSION

Transformation of waste into potential economic resource is a major challenge. Improper disposal of waste human hair could adversely impact environment and health. Seeking to extract the value from waste hair, which would otherwise be burned or buried as waste, developed the scope and opportunity for the booming industry of waste hair reprocessing with high economic turn-over. As it is labour intensive, the growth of such cottage industry involve poor people who do not have any environmental perspective or concept of personal health and hygiene. Various applications of waste human hair (Gupta, 2014) highlight the need of skilled labour to maintain such large scale economy evolved around 'human hair'. It is indeed important to link both human health and environmental health through effective and environment safe management of waste hair.

A number of studies have been conducted on hair dressers (Rickes et al., 2010; Bradshaw et al., 2011; Mendes et al., 2011; Dulon et al., 2011; Jung et al., 2014; Hassan and Bayomy, 2015) that identified health risks (respiratory, allergic and pain related symptoms) including cancer from all over the world, like UK, Portugal, Brazil, Korea, Germany and Egypt. Harling et al., 2010, performed a robust meta-analysis on 42 studies suggesting risk of bladder cancer particularly when hair dressers were more than 10 years in their occupation. Lung cancer risk among hair dressers were identified by several studies (Hashemi et al., 2010). Pooled analysis by (Olsson et al., 2013) identified that smoking significantly increased the risk of lung cancer. It is noteworthy to mention here that many hazardous dyes has been phased out and replaced by less harmful chemical substances in salon during direct human application, assuming reduced occupational health hazards in recent times. However, our study deals with the rural workers who use waste hair for making wigs. The workers are involved in this occupation for more than 10 years with at least 7 hours/ day, without following any safety guidelines or using any personal protective equipment. Comparing the existing literature on hair dressers, we observed wig processing workers had higher health problems in respiratory, allergic manifestation as well as musculoskeletal pain compared to the studies on hair-dressers done by other research groups reported so far (Table 8).

According to Dembe et al., (2005), duration of job held especially greater than 10 years is a significant cause of occupational risk. Quite noticeably, female workers are prevalent in this occupation, often found to be associated with their children; as the reprocessing procedure is based on a concept of 'work from home'. Pregnant and lactating mothers are also involved in this occupation and thus posing an adverse effect on the developing fetus and infants. On the other hand most of the male workers (100 % of male in our study population) were addicted to tobacco products, which might have a synergistic effect on their occupational health, as use of tobacco is already found to be a potential confounder for urinary bladder and lung cancer risk (Freedman et al., 2011).

Hair processing (fine hair particles as well as chemicals used) may be considered as the primary reason for the respiratory problems whereas the cleaning and cutting work involving repetitive manual movement may increase the risk of musculoskeletal disorder related problems. Workers are heavily exposed to various chemicals, either through contact (dermal exposure) or inhalation (volatile and particulate form) during mixing of the ingredients and also at the time of application. The gaseous substances and particulate matter once inhaled irritate the respiratory tract and cause respiratory troubles. Most of them are considered toxic for human, like Paraphenylene diamine (P-pD) and other ammonia compounds (Ferrari et al., 2005; Khumalo et al., 2006). Prolonged exposure to these chemical hazards might create deleterious health effects including risk of cancer (Gago et al., 2001; Rauscher et al., 2004).Workers are also at a risk from use of p-PD (potential carcinogenic dye) in bare hand during their work process. The route of exposure of pPD is via skin which might be absorbed through systemic circulation and cause nuclear anomalies in exfoliated urinary epithelial cells. We found significant increase of alveolar macrophages (a cellular defense mechanism) from the toxic insult. Increase in AM was noticed in individuals with severe air pollution (Lahiri et al., 2000; Yuriko et al., 2013), strongly emphasizing the cause-effect relationship with particulate matter. MN analyses from exfoliated cells were used universally by different groups of researchers as it is a simple as well as sensitive method to identify cytogenetic damage (Rickes et al., 2010, Ghosh et al., 2006).

ERCC2 and its association with DNA damage has documented in several studies (Duell et al 2000, Clarkson and Wood 2005). More specifically, individuals with *ERCC2* codon 751 Lys/Lys polymorphism show increased susceptibility towards arsenic induced premalignant skin lesions (Banerjee et al., 2007), esophageal squamous cell carcinoma (Li et. al., 2013) and lung cancer (Li et al., 2014). The present study identifies occupational hazards and associated health risks among the workers. Moreover, we studied the role of individual genetic susceptibility of the study population to relate the cytogenetic damage. The present study confirms genotype (codon 751 Lys/Lys)-phenotype (higher MN formation) correlation irrespective of exposure background and the prevalence of risk genotype (Lys/Lys) was significantly higher among workers. The study inferred potential cancer risk of the population having risk genotype.

TABLE 8: Comparative analysis of health problems between hair processing workers with existing literatures on Hair-dressers

Study Population		**Respiratory problems**		**Allergic Manifestation**			**Musculoskeletal problem**				**Ref**
Waste hair reprocess-sing workers	**N=502**	**Cough-ing**	**Asthma**	**Derma-titis**	**Eye irritati-on**	**Nasal problem**	**Neck pain**	**Should er pain**	**Elbow pain**	**Wrist & hand pain**	**Present paper**
		50%	15%	31%	37%	34.2%	64%	57.2%	37.8%	41.8%	
Hairdres-ser	N=80	25%	16.3%	17.5%	20%	22.5%	36.3%	38.8%	21.3%	41.3%	Hassan & Bayo my, 2015
	N=50	30%	29%	NA	NA	NA	NA	NA	NA	NA	Hashmei, N.et al-2010
	N=50	NA	17%	21%	43%	37%	NA	NA	NA	NA	Mendes et al-2011

V. CONCLUSION

Waste burden is increasing at an alarming rate and the global challenge lies in the transformation of waste to resource. Increased awareness about various utilization of human hair ensures its acceptability as a potential 'resource' and thus will encourage the rise of modern technologies (with environment and health safeguards) to facilitate entrepreneurial need considering workers' health and socio-economic benefit. It is important to know the safe working procedures as otherwise occupation hazards along with individual susceptibility could cause significant health problems. It is also important to ensure environmental well being, otherwise disposal of waste can increase disease risk and affect ecosystem by contaminated soil and water-bodies. The acceptance of the knowledge of the potentiality of hair as a resource and "not waste" will encourage workers to follow healthy practices; scientists to develop better technology and entrepreneurs to get a booming market. As our endeavour, we made people aware of hygiene measures, provided PPEs to reduce exposure to hair waste and chemicals, demonstrated the ways of safe disposal of waste and hope these are the best preventive tools, which will have a positive impact on prospective studies in future.

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Author contribution

Field studies: K.M. Screening of study participants and biological sample collection: Dr. S.B. Experiment: K.M. and T.S., Acquisition of data and analysis: K.M. and T.S., Statistical analyses: S.D; Manuscript writing: T.S and K.M.; Editing and Critical Analysis: Dr. P.B. and Dr. S.B. Supervised by Dr. P.B.

Conflict of interest

The authors declare that they have no conflict of interest.

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