

# DNA damage and TNF $\alpha$ cytokine production in hairdressers with contact dermatitis

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The present work was undertaken to study in hairdressers exposed to several irritants and allergens (prevalently hair-dyeing) and affected by hand contact dermatitis the possible correlation between exposure and direct-oxidative DNA damage, production of tumour necrosis factor alpha (TNF $\alpha$ ) and allergic inflammatory disease. We evaluated in 19 hairdressers with hand contact dermatitis, 14 allergic contact dermatitis (ACD) and 5 irritant contact dermatitis (ICD) and in a selected control group TNF $\alpha$  serum levels by ELISA and direct-oxidative DNA damage by Fpg (formamido-pyrimidine-glycosylase)-modified Comet test on blood. Hairdressers were divided on the basis of number of hair-dyeing carried out weekly into 2 groups: low-exposure (<60 hair-dyeing/week) and high-exposure hairdressers ( $\geq 60$  hair-dyeing/week) that reflect also the exposure to other allergens and irritants and 2 different tasks (hairdressers and apprentice hairdressers, respectively). Serum levels of TNF $\alpha$  in hairdressers with ACD were significantly higher than controls with a correlation to exposure level. Significant DNA damage in ICD hairdressers with higher exposure as compared to controls was found. These findings suggest that occupational exposure can induce in hairdressers, particularly ICD, DNA damage, increase the TNF $\alpha$  levels particularly in ACD and induce allergic sensitization, suggesting a relationship between direct-oxidative DNA damage, TNF $\alpha$  production and allergic inflammatory disease.

**Keywords:** direct-oxidative DNA damage; hairdressers; hand dermatitis; TNF $\alpha$ . © Blackwell Munksgaard, 2005.

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Contact dermatitis is the most common occupational skin disorder. This disease consists essentially of irritant contact dermatitis (ICD), found in approximately 80% of cases, and allergic contact dermatitis (ACD) found in the remaining 20% (1). Hairdressers are particularly at risk because they are occupationally exposed to many synthetic and natural chemicals, even strongly allergenic or irritants, to water and soap (which can act chronically as weak irritants), without specific protection because of type and methods of their work. In fact, hairdressing shops are often craft-scale establishments, not inspected enough, with rapid microclimatic changes during working time. Moreover, hairdressers rarely use individual protective measures and hardly receive instruction on risks of exposure. This occupational exposure was classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic (Group 2A) (2). The daily exposure to

allergens and irritants induces frequently in this occupational category hand contact dermatitis with ICD or ACD origin. The average incidence rate of hand contact dermatitis reported in a study on hairdressers was 32.8 cases per 100 person-years (3). The ICD, which is an inflammatory disease caused by skin contact with irritants, inflicts damage through a non-immunologic mechanism and seems to be, among hairdressers, more frequent in apprentices due to their prolonged contact with water and shampoos (4). ACD is a reaction of the immune system in response to skin contact to the culprit substance and is frequently reported in hairdressers as indicated in an Italian study on 302 hairdressers with dermatitis that found the presence of ACD in the 60.9% of cases (4).

In this disease, the cytokines [tumour necrosis factor alpha (TNF $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6] play a key role. In particular, the TNF $\alpha$  is regarded as a critical mediator of the allergic

response (5).  $\text{TNF}\alpha$  is upregulated in response to experimental contact allergens (6). When the  $\text{TNF}\alpha$  production is high, it enters in haematic circle where it works by endocrine mechanisms at tissue level developing several allergic inflammatory diseases such as dermatitis. Hair dyes are a frequent cause of ACD among hairdressers and the *p*-phenylenediamine (PPD) is the principal contact allergen found in conventional hair dyes, but we have little information about their mechanism of action at cellular level. An *in vitro* study of the interactions between allergens and keratinocytes in the pre-immunological phase of contact sensitization indicated that oxidative stress was induced by exposure to PPD, suggesting a relation between this type of cellular effect and the development of allergic inflammatory disease (7). Another study, *in vivo* on guinea pigs exposed to PPD, found oxidative stress, with an increase in lipid peroxidation and raised levels of antioxidant enzymes and histamine and allergic dermatitis (8). Occupational contact dermatitis from PPD is common among hairdressers and is reported in 19%–35% of exposed subjects (9, 10). Moreover, hair-dye exposure was found to be related to an increased risk of bladder cancer (11), indicating also a potential carcinogenic effect for these substances.

We designed this study to clarify in ACD and ICD hairdressers whether there is any relation between hairdressers occupational exposure, increased  $\text{TNF}\alpha$  serum levels and allergic inflammatory disease with the aim to investigate the events that might lead to the development of contact dermatitis. Moreover, we investigated the possible carcinogenic effect of hairdressers occupational exposure evaluating early direct or oxidative DNA damage with the aim to furnish useful indication in terms of risk assessment.

## Patients and Methods

### Patients

We recruited 19 hairdressers (13 women and 6 men) with chronic eczematous contact dermatitis of the hands among patients referred to the Department of Allergology of S. Gallicano Hospital in Rome. The hospital Ethical Committee and the ISPESL Scientific Committee approved the human study. All subjects gave informed consent.

Anagraphic, clinical, working information and lifestyle habits (smoking, dietary habit and alcohol consumption) were obtained from a questionnaire administered by specialized medical personnel. The hairdressers consisted of 8 smokers and 11 non-smokers with a mean age of 25.6 years ( $\pm 8.7$  SD) all living in urban area and no

alcohol consuming. The subjects were divided on the basis of number of hair-dyeing carried out weekly into 2 groups: low-exposure hairdressers ( $<60$  hair-dyeing/week) and high-exposure hairdressers ( $\geq 60$  hair-dyeing/week) in relation to the distribution of hair-dyeing/week number that showed 2 ranges of continuous values (20–40 and 60–80). These 2 exposure groups reflected 2 different tasks (hairdressers and apprentice hairdressers, respectively).

Only 3/19 hairdressers belonging to the low-exposure group did not use gloves. To diagnose the origin of dermatitis, these patients were patch-tested with a standard series and with a limited series of occupational relevance. Patches were applied for 48 hr and read at 48 hr and 96 hr. The presence of palpable erythema was taken to indicate a positive patch-test reaction. We recruited a control group of 19 healthy subjects employed in the administrative area consisting of 14 women and 5 men (9 smokers and 10 non-smokers) with a mean age 31 years (SD 4.6). The control group was selected to match the exposed group for sex and lifestyle habits (smoking, dietary habit and alcohol consumption).

### Methods

Whole venous blood samples were taken from patients and controls by venipuncture by specialized medical personnel, in sterile heparinized disposable syringes at Allergology Department of S. Gallicano Hospital, and transferred within 2 hr, at room temperature and in the dark, to the laboratory where the analyses were performed. Lymphocytes were isolated on a Ficoll-based density gradient and immediately used for Comet test, while the serum samples were stored at  $-80^\circ\text{C}$  to be analysed successively.

At the moment of analysis, serum samples were defrosted and an ELISA kit (Roche Diagnostics GmbH, Mannheim, Germany) was used to measure  $\text{TNF}\alpha$  levels in hairdressers and controls.

We used formamido-pyrimidine-glycosylase (Fpg)-modified Comet assay to evaluate oxidative DNA damage which use Fpg enzyme, a glycosylase that recognizes and specifically cuts the oxidized bases principally 8-oxoguanine from DNA producing apurinic site converted in breaks by the associated AP-endonuclease activity. Therefore, these breaks can be detected by Comet assay and give a measure of DNA oxidative damage, enabling us to detect moderate but still appreciable damage even at low exposures. We followed the procedure of Collins (12), with minor modifications. 2 blond gel films were prepared for each case (1 to be treated without and the other with Fpg) allowing the

detection of direct DNA lesions (single – double strand breaks and alkali-labile sites) and oxidative DNA damage, respectively (12). On top of each film, about 20 µl of lymphocytes (isolated by Ficoll from peripheral blood and suspended in 1 ml of PBS) was layered, mixed with 70 µl of low-melting agarose 0.7% in PBS at 37°C. Then they were bathed in lysis solution (2.5 M NaCl, 100 mM Na<sub>2</sub>EDTA, 10 mM Tris with 1% Triton X-100 and 10% DMSO added fresh) and kept in the dark for 1 hr at 4°C.

The slides were washed 3 times in enzyme buffer (50 mM Na<sub>3</sub>PO<sub>4</sub>, 10 mM EDTA, 100 mM NaCl, pH 7.5), drained and incubated with 50 µl of either buffer or Fpg (1 µg/ml in enzyme buffer) in the dark for 30 min at 37°C.

The slides were placed in a horizontal gel electrophoresis tank filled with fresh alkaline buffer (1 mM Na<sub>2</sub>EDTA and 300 mM NaOH, pH 13) for 40 min at 4°C to allow denaturing and unwinding of the DNA and the expression of alkaline-labile sites.

Electrophoresis was done in the same buffer at 25 V and 300 mA for 30 min to allow the fragments of damaged DNA to migrate towards the anode. The slides were then washed 3 times with Tris – HCl 0.4 M for 5 min and stained with 50 µl ethidium bromide (10 µg/ml).

Slides were examined by eye at 200× magnification under a fluorescence microscope. An undamaged cell appeared as a nucleoid and a cell with damaged DNA as a comet. Images of 50 randomly selected comets stained with ethidium bromide were acquired and analysed from each sample with specific image analyzer software (Delta Sistemi, Rome, Italy).

Measurements of comet parameters were: % DNA in the tail, tail length and tail moment (the product of relative tail intensity and length, used as a measure of DNA damage). For each subject, we calculated the mean tail moment for 50 comets from Fpg-treated cells (TMenz) that furnishes an indirect measurement of oxidative

DNA damage. We also calculated the mean tail moment for Fpg-untreated cells (TM), which indicates direct DNA damage. For statistical analysis, ANOVA multivariate and non-parametric tests for non-normal distribution (Kruskal–Wallis and Mann–Whitney *U*-test) were used and a  $P \leq 0.05$  was considered significant.

## Results

14 of 19 hairdressers gave a positive patch-test reaction to limited series of substances of occupational relevance, principally PPD (all subjects) and *p*-toluylenediamine (7/14 subjects) contained in hair-dyeing, and the resulting diagnosis was ACD. The remaining 5 patients were negative to patch tests and for them the diagnosis was ICD.

The high-exposure group was characterized with respect to low-exposure group by a lower mean age value (21.6 versus 31.12), a prevalence of females (9/11 versus 4/8 subjects), a lower number of smokers (3/11 versus 4/8 subjects) and a prevalence of hairdresser apprentices (10/11 versus 0/8).

Quantitative assay of TNFα in serum gave a mean concentration of 27.71 pg/ml in the hairdressers with ACD and 18.64 pg/ml in those with ICD, compared to 12.30 pg/ml in controls (Table 1), and the Kruskal – Wallis test showed a  $P = 0.054$  near to statistical significance but which was not reached probably because of the small sample size. Mann – Whitney *U*-test showed a significant difference for TNFα mean values between exposed subjects and controls ( $P = 0.034$ ). Moreover, Kruskal – Wallis test showed TNFα mean values among ICD, ACD and controls significantly different in relation to exposure level ( $P = 0.045$ ). The Comet test results, summarized in Table 1, show the highest mean TM and TMenz values in ICD group (22.48 and 36.48, respectively). ANOVA multivariate analysis showed significant differences of TM ( $P = 0.013$ ) and TMenz ( $P = 0.025$ ) values. The two-way ANOVA analysis was used to

Table 1. Tumour necrosis factor alpha (TNFα) serum levels and mean tail moment values obtained by Comet test on allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD) hairdressers groups each considered altogether and subdivided into high-, low-exposure and control groups

|                                            | TNFα (pg/ml) | Mean TM     | Mean TMenz   |
|--------------------------------------------|--------------|-------------|--------------|
| ACD hairdressers ( $n = 14$ )              | 27.71        | 12.83 ± 5.3 | 24.97 ± 12.1 |
| High-exposure ACD hairdressers ( $n = 8$ ) | 35.4         | 13.20 ± 5.5 | 26.4 ± 15    |
| Low-exposure ACD hairdressers ( $n = 6$ )  | 17.48        | 12.35 ± 5.5 | 23.08 ± 5.9  |
| Control group ( $n = 19$ )                 | 12.30        | 14.32 ± 4   | 22.16 ± 4.3  |
| ICD hairdressers ( $n = 5$ )               | 18.64        | 22.48 ± 12  | 36.48 ± 17.1 |
| High-exposure ICD hairdressers ( $n = 3$ ) | 10           | 29.56 ± 9   | 47.3 ± 7.5   |
| Low-exposure ICD hairdressers ( $n = 2$ )  | 31.6         | 11.86 ± 6.1 | 20.26 ± 13   |
| Control group ( $n = 19$ )                 | 12.30        | 14.32 ± 4   | 22.16 ± 4.3  |

TM, mean tail moment value of 50 comets from cells untreated with Fpg enzyme; TMenz, tail moment of 50 comets from Fpg-treated cells.

compare the effect on the mean values of TM and TMenz with respect to the exposure level and presence (ICD and ACD) or absence (controls) of disease, and demonstrated a statistically significant difference between TM and TMenz values and exposure level ( $P = 0.002$  for TM and  $P = 0.003$  for TMenz) with the highest mean TM and TMenz values in the high exposed ICD group. No significant associations between presence of direct or oxidative DNA damage and age or smoking habits were found.

### Discussion

TNF $\alpha$  is upregulated in response to experimental contact allergens (6) and is regarded as a critical mediator in the pathogenesis of ACD (5). The production of TNF $\alpha$  is regulated at the transcriptional and post-transcriptional levels (13). Our findings related to TNF $\alpha$  levels of ACD group showed a correlation with the exposure level; in fact higher values of TNF $\alpha$  in the high exposure were found. These results suggest a relationship between production of this cytokine and allergic contact dermatitis.

Some studies showed a carcinogenic effect of hair dyes in hairdressers, but a few studies are actually available on their mechanism of action at cellular level. In particular, an *in vitro* and an *in vivo* study performed on PPD, which is the most frequent chemical contained in hair dye, showed the induction of oxidative stress with an increase in lipid peroxidation and raised levels of antioxidant enzymes after exposure to PPD and suggested a relationship between oxidative stress and development of allergic inflammatory disease (7, 8). New methods for studying DNA oxidative damage and repair (14) hold out promise for further clarifying the mechanisms of action of hair dyes also in relation to their potential carcinogenicity. In the last years, the Comet assay has become an important tool to assess genotoxic damage of xenobiotics both in studies on possible mechanisms of action of genotoxic and carcinogen agents and in human biomonitoring studies on exposed populations (15–17). Comet assay is a rapid and sensitive technique that allows to identify early direct DNA damage, early oxidative DNA damage evaluated by Fpg modification, repairable injuries or alkali-label sites.

Our results, obtained by Fpg Comet test, indicated that hairdressers exposure can induce in ICD group direct and oxidative DNA damage; in fact a relationship between the exposure level (high and low) and DNA damage was found. The presence of oxidative DNA damage in highly exposed ICD subjects could be due to a direct

induction of oxidative damage by irritants and hair dyes through reactive oxygen species generation and inducible nitric oxide synthase induction together with inflammatory response to exposure. The allergen and irritant-induced DNA damage might be an essential step in the preimmunologic stage of contact sensitization, confirming the suggestion that these substances act at cellular level. The use of Fpg-modified Comet assay that gives a specific and accurate measure of DNA damage allowed us to detect also moderate but still appreciable direct and oxidative damage even at low exposures, confirming the hypothesized carcinogenic action of hair dyes.

To conclude, it would appear that increased production of TNF $\alpha$ , hair-dye exposure and ACD are all related, suggesting a possible relationship between allergen exposure, cytokine induction and allergic disease development. Moreover, our study shows a possible association between presence of direct-oxidative DNA damage and occupational exposure in ICD hairdressers. The presence of DNA damage in ICD could suggest a higher susceptibility of these subjects to develop genotoxic and oxidative effects by chronic exposure to mixture of substances used in hairdressing work, whose probable carcinogenicity has been indicated by IARC. It obviously calls for further studies in the field involving larger numbers of ICD hairdressers.

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