INTERNATIONAL JOURNAL OF OCCUPATIONAL AND ENVIRONMENTAL HEALTH
2018, VOL. 24, NOS. 3–4, 126–133
https://doi.org/10.1080/10773525.2018.1523860

Bio-monitoring of DNA damage in matchstick industry workers from Peshawar Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Background: Safety protocols are usually neglected in most of the matchstick industries rendering the laborer prone to various occupational hazards.
Objective: The present study highlights DNA damage among matchstick factory workers (n = 92) against a control group (n = 48) of healthy individuals.
Methods: Genotoxicity was measured in peripheral blood lymphocytes of the test subjects using a Single Cell Gel Electrophoresis assay (SCGE/comet assay).
Results: Our results substantiate a high *Total Comet Score* (TCS) for factory workers (74.5 ± 47.0) when compared to the control group (53.0 ± 25.0) ($P \leq 0.001$). Age and duration of occupational exposure had no significant effect ($P > 0.05$) on TCS value. As for job function, the TCS value was greatest in sweepers (91.0 ± 56.1) and lowest in box-making operators (26.0 ± 25.0) indicating that waste disposal poses the higher risk of DNA damage.
Conclusions: Our study corroborates that matchstick chemicals can potentially damage the DNA of exposed subjects.

ARTICLE HISTORY
Received 19 December 2016
Revised 12 September 2018
Accepted 12 September 2018

KEYWORDS
Genotoxic effect; DNA damage; matchstick industry; comet assay

Introduction

Pakistan is one of the main producers of Safety Matches in the world. The country has exported matches since the 1990s to a wide range of world markets including the African continent, the Middle East, the Far East, Europe, and Latin America. Revenue generated from safety match exports exceeds 40 million US dollars per annum [1]. However, little attention has been given to the occupational hazards associated with the matchstick industry. A labor workforce is a valuable asset to all industries, and determines a region's productivity and economic growth if the best protection protocols for the workforce are in place [2]. However in developing countries, lack of protective legislation, low standards in corporate systems and governance, high labor-intensive character, and inadequate infrastructure are factors that combine to work against labor safety [3].

Exposure to chemicals used in matchstick preparation may put factory workers specifically at a greater risk [4,5]. For instance potassium chlorate, used in the head of the match, and red phosphorus in striking surface are associated with numerous health disorders such as inflammation of respiratory membranes, nausea, vomiting, diarrhea, cachexia, anemia, cyanosis, coma, anuria, convulsions, jaundice, kidney and liver damage, skin irritation, and eye irritation [6]. Occupational hazards presented by match industry are well documented in recent history [7]. Phossy jaw was noticed in workers associated with inhalation of fumes of white phosphorus [8]. The condition is characterized by jaw bone disease and phosphorus necrosis often seen in the matchstick industry. Inhalation of phosphorus oxide is very harmful as it causes irritation, severe epigastric pain, jaundice, vomiting, and depression. It can also cause a headache, anemia, dyspepsia, loss of appetite, slowness of wound healing, and albuminuria [8,9]. Phosphine gas, produced as a result of a reaction of red phosphorus, water vapors, and oxygen at room temperature is similarly extremely toxic. Inhalation causes shortness of breath, chest pain, and high respiratory rate [9].

Studies have shown that red phosphorus, which is one of the most important ingredients of matchsticks, is a weak mutagen [10]; a substance that interferes with the integrity of the DNA molecule and its replication. Abnormal DNA replication is a primary cause of mutations related to pathological disorders including cancer.

A number of techniques such as counting sister chromatid exchanges, other chromosomal aberrations and the presence of micronuclei are commonly used for evaluating the environmentally induced genetic damage. However, these methods are time consuming, costly, and require proliferating cells, so the use of comet assay or single cell gel electrophoresis (SCGE) for genotoxicity studies have greatly increased during

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the past few decades [11–13]. The comet assay is a sensitive and rapid method for assessment of DNA damage at the single-cell level and it provides information on the detection of DNA single-strand breaks, double-strand breaks, and alkali-labile sites [14–16].

In this perspective, the present study investigates the extent of DNA damage in workers of several matchstick industries operating in the Hayatabad Industrial Estate of provincial capital, that is Peshawar of Khyber Pakhtunkhwa. Most of the workers targeted during the present study were uneducated and seldom had knowledge about standard safety protocols. The study was aimed at highlighting the potential genotoxic effects of workplace exposures as well as increasing awareness among labor by educating them about common occupational threats and standard safety protocols.

Materials and methods

Study population

A total of 92 workers from nine match factories of Peshawar, were included in the study. Workers were included in this study who had at least two years working experience in the matchstick industry. The mean age of the exposed group was 39.7 years ±12.7. The mean period of relevant employment was 13.7 years ±8.1, and mean daily exposure was 10.3 ± 1.4 h. Among the factory workers, 32 subjects were classified as smokers while 60 subjects were nonsmokers. The control group consisted of 48 healthy individuals with 33.1% university employees, 25.6% shopkeepers, 41.3% unemployed people selected from the same region with a mean age of 36.2 years ±13.2 with no exposure to matchstick fumes or any other occupational exposure to genotoxicants (Table 1). The study was approved by the Ethical Committee, University of Peshawar.

Questionnaire

Relevant information, including age, gender, daily work hours, years of exposure, type of job, use of protective measures, preexisting diseases, and smoking habits were collected by questionnaire from the study populations. Informed consent was signed by all the study participants. Blood samples were collected from the subjects at their workplace on working days.

Table 1. Characteristics of the study population.

	Exposed group	Control group
Number of subjects	92	48
Mean age (years)	39.7 ± 12.7	36.18 ± 13.20
Mean years of exposure	13.7 ± 8.1	.....
Mean daily exposure (hours)	10.3 ± 1.4	.....
Tobacco addiction		
Non-smokers	60 (65.2%)	22 (45.8%)
Smokers	32 (34.8%)	26 (54.2%)

Blood sample collection and lymphocyte isolation

In a disposable syringe, approximately 5 mL of blood was collected from each subject and was transferred to EDTA tube. The samples were labeled and carried to the laboratory within 2–3 h to perform the comet assay. Lymphocytes were isolated from the blood by Histopaque-1077 density gradient centrifugation and washed in phosphate buffered saline. Trypan blue dye was used for testing cell viability, which was greater than 90% in all cases.

Alkaline comet assay

The alkaline comet assay technique was performed as described by Singh et al., 1988 [17]. This assay allows assessment of the total of DNA single-strand and double-strand breaks as well as alkali labile DNA modifications. Duplicate comet assay slides were prepared for each sample. For the preparation, conventional glass microscope slides were dipped into molten normal melting agarose (NMA) (0.7 %), laid in a tray to air dry and then wiped from the underside to remove the extra agarose. Slides were generally prepared 1 day before use, labeled and then stored at room temperature. 15 µl of cell suspension was mixed with 70 µl of low melting point agarose (LMPA) (0.7 %), spread on top of precoated slides and kept at 0°C for 5 min with a coverslip. After that the coverslip was removed, the second layer of 85 µl LMPA was added to fill any residual holes and agarose was set at 0°C for 5 min, with a coverslip in place.

Cell lysis

After solidification, the coverslips were removed and slides were gently immersed in a freshly prepared cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10) with 1% Triton X-100 and 10% DMSO added just before use for at least 2 h at 4°C.

Electrophoresis and neutralization

After cell lysis, the slides were immersed in electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13) and left for 20 min to allow the denaturing of DNA and conversion of alkali-labile sites to breaks. The slides were then subjected to electrophoresis for 25 min at 300 mA and 25 V. To prevent any kind of unintentional DNA damage, the slides were protected from direct exposure to

Table 2. Mean frequency of each comet class per 100 cells (±standard deviation) and overall mean of total comet score (±standard deviation) of the exposed subject and control group.

Comet class	0	1	2	3	4	TCS
Exposed subject	56.7 ± 23.8	23.5 ± 16.4	9.1 ± 8.6	5.1 ± 6.3	4.4 ± 4.4	74.5 ± 47.0*
Control subject	72.4 ± 11.4	13.6 ± 6	6.3 ± 4.3	4.0 ± 3.9	3.7 ± 3.2	53.0 ± 25.0

*Difference significant relative to control group at $P \leq 0.001$ (Student's *t*-test, two-tail), TCS: Total comet score

light. The steps were conducted at 4°C. After electrophoresis, the slides were neutralized by washing three times (400 mM Tris, pH 7.5) for 5 min each.

Staining, scoring, and visualization of slides

The slides were stained with 70 µl Acridine orange dye (20 µg/ml) and kept for 5 min, coverslips were placed on the slide, and the samples were viewed by fluorescence microscopy (Nikon Eclipse 80 i equipped with 450–490 nm excitation filter). In order to calculate DNA damage, 100 cells per sample were chosen randomly and analyzed visually according to comet appearance. Five classes, i.e. from class 0 (no DNA damage) to class 4 (maximum DNA damage) give sufficient declaration. Visual scoring is a reliable, simple, and rapid method for scoring the comets [18]. Total comet score (TCS) was then calculated according to the formula, TCS = 0(n) +1(n) +2(n) +3(n) +4(n), where "n" indicates number of cells in each class, thus the overall score for each slide was therefore between 0 (undamaged) and 400 (maximum damaged), as referred by Collins [18] and reported in our previous publications [19–21].

Statistical analysis

Statistical analysis was performed, using SPSS V.20.0. Mean and standard deviation values of the data were determined. The comet assay data were analyzed using Student's t-test (two-tail), where P value was kept at 0.05 for statistical significance. The correlation was calculated for the duration of occupational exposure and TCS by using the Spearman correlation test. Comparisons among natures of the task in the factory were carried out by one-way analysis of variance (ANOVA) with the level of significance set at $P < 0.05$.

Results

The characteristic features of the investigated matchstick worker population are shown in Table 1.

Significantly greater DNA damage was observed in the matchstick factory workers (TCS = 74.5 ± 47.0) compared with that observed in the control group (TCS = 53.0 ± 25.0, $P < 0.001$) (Table 2). In addition, comet class 3 (5.1 ± 6.3 cells) and class 4 (4.4 ± 4.4 cells) were observed more frequently in matchstick factory workers than in the control group (comet class 3 = 4.0 ± 3.9 and comet class 4 = 3.7 ± 3.2 cells, respectively). The opposite results were observed with undamaged cells; comet class 0 was observed more frequently in the control group (72.4 ± 11.4) as compared with the matchstick workers (56.7 ± 23.8 cells) (Table 2 and Figure 1).

Men who worked as matchstick factory workers for >11 years had the highest mean comet score (77.7 ± 45.7), though the duration of occupational exposure was not correlated with comet score ($r = -0.060$; $P > 0.05$) (Table 3 and Figure 2).

Concerning age, there was no significant effect ($P > 0.05$) on TCS in the factory workers (Table 4 and Figure 3), that is DNA damage was the same for young and older workers.

When Matchstick factory workers were evaluated according to their job function in the factories, the mean TCS observed in sweepers (91.0 ± 56.1) was significantly higher ($P < 0.05$) and box making operators (26.0 ± 25.0) was significantly lower ($P < 0.05$) than that observed in rest of the factory works (Table 5 and Figure 4). The nature of chemical exposures in each of the task areas is shown in Table 6.

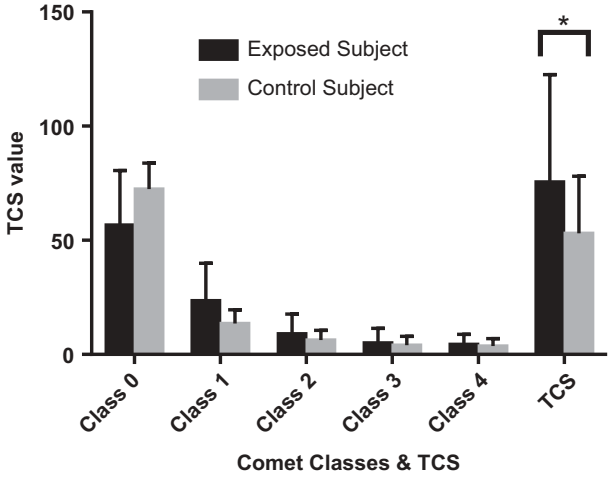

Figure 1. Mean frequency of each comet class and TCS of exposed and control subjects. Values are expressed in Mean ± S.D. *$P < 0.001$ statistically significant compared with control.

Table 3. Comet score according to years of exposure.

Years of occupation	*N* (%)	TCS
≤10	39 (42.4)	71.5 ± 47.4
≥11	53 (35.9)	77.7 ± 45.7
Total	92	74.5 ± 47.0

$r = -0.060$, $P > 0.05$ (Spearman correlation test)

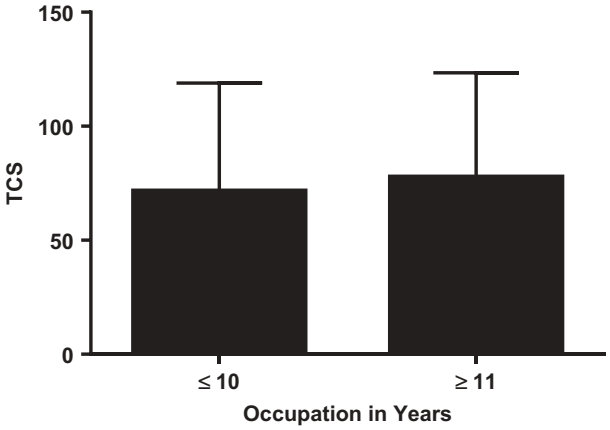

Figure 2. Correlation between occupational exposure and TCS. Values are expressed as Mean ± S.D. Correlation is negative and non-significant $r = -0.060$, $P > 0.05$.

Table 4. Influence of age on total comet score (mean ± SD).

Subjects	TCS
Control group	
≤40 years old (n = 24)	48.1 ± 20.8
>40 years old (n = 24)	61.5 ± 30.0
Exposed group	
≤40 years old (n = 54)	72.8 ± 46.4*
>40 years old (n = 38)	74.7 ± 44.1*

*Difference nonsignificant $P > 0.05$ relative to control groups (Student's t-test, two-tail)

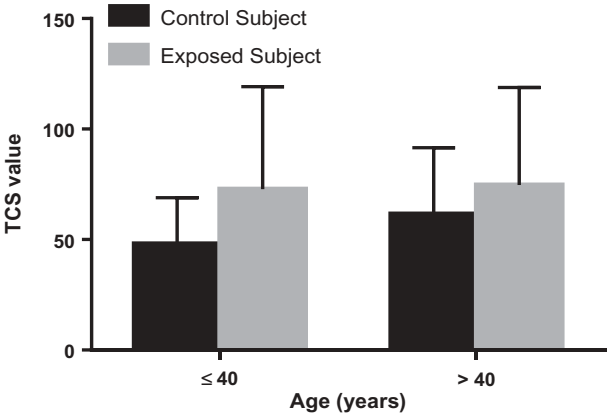

Figure 3. Effect of age on TCS of exposed and control groups. Values are expressed in mean ± S.D.

Table 5. Distribution of TCS according to task in the factory.

Nature of task	N	TCS
Indirect exposed employee	24	81.2 ± 45.7
Directly exposed employee		
Sweeper	5	91.0 ± 56.1*
Box filling operator	15	86.4 ± 52.6
Chemical preparatory	5	80.5 ± 39.5
Chopping or Splint operator	7	78.5 ± 54.5
Mechanic	9	76.0 ± 46.3
Dye cutting machine operator	2	75.2 ± 46.6
Samplex operator	9	59.3 ± 32.0
Box making operator	6	26.0 ± 25.0*
Control	48	53.0 ± 25.0

*$P < 0.05$, One way ANOVA (F-test)

Smoking habits had a significant effect ($P < 0.05$) on TCS value among the exposed and control groups (Table 7 and Figure 5).

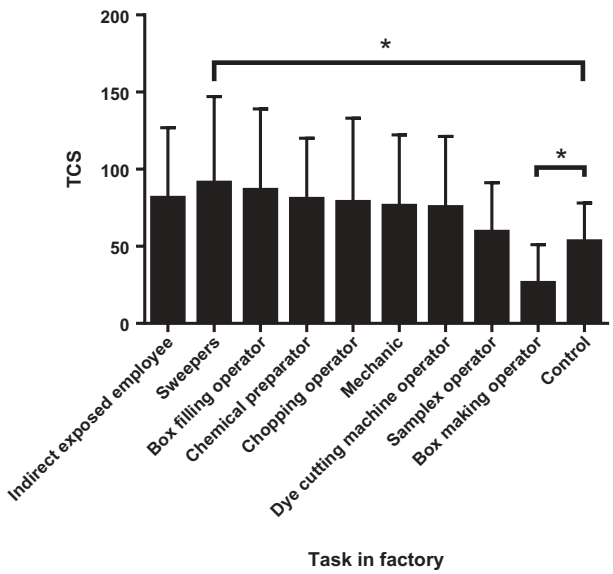

Figure 4. Distribution of TCS according to the nature of the task in the factory compared with TCS of the control group. Values expressed in Mean ± S.D. *$P < 0.05$ statistically significant compared with the control group.

Discussion

The present study was conducted to determine DNA damage among matchstick factory workers. Our results reveal a significantly higher TCS of exposed group than that of the control group ($P < 0.05$). This higher TCS score of the exposed group suggests that the matchsticks chemicals are a cause of DNA damage in factory workers. Several studies carried out previously support this assumption. For instance a recent study conducted on mice revealed that exposure by instillation to low doses of carbon black (an important constituent of match box) is followed by DNA damage [22]. Similarly, Phosphine (PH_3) had been described to generate oxidative DNA damage in mouse hepatoma Hepa 1c1c7 cells *in vitro* and in rat liver and brain *in vivo* after an intraperitoneal treatment [23,24]. Other studies showed that it induces oxidative damage in animals and is a respiratory inhibitor [25]. It stimulates the production of hydrogen peroxide and reactive oxygen species (ROS), hinders the activities of cytochrome c oxidase, catalase, and peroxidase, and elevates superoxide dismutase (SOD) [26,27]. Likewise, in a micronucleus analysis performed on circulating RBC and on bone-marrow polychromatic and normal chromatic red blood cells of female rats, the red phosphorus-butyl rubber smoke was found to be a weak clastogenic [6]. Our study suggests that occupational exposure to airborne chemicals in matchstick manufacturing can damage the DNA of somatic cells (lymphocytes) and this could be an initial step in the process of chemical carcinogenesis. Exposure to genotoxic compounds such as dust, asbestos fibers and other airborne

Table 6. Nature of chemical exposures in each of the task areas.

Task area	Nature of chemical exposure
Indirectly exposed employee	Fumes and dust of red phosphorus, potassium chlorate, phosphine gas, phosphorus oxide
Sweepers	Cleansers like phenyl and bleach, red phosphorus, potassium chlorate, phosphine gas, phosphorus oxide
Chemical preparatory	Red phosphorus, potassium chlorate, phosphine gas, phosphorus oxide
Box filling operators	Red phosphorus, potassium chlorate
Chopping or splint operator	Red phosphorus, potassium chlorate
Mechanic	Red phosphorus, potassium chlorate, lubricant oils etc
Dye cutting machine operator	Dye chemicals, red phosphorus, potassium chlorate, phosphine gas, phosphorus oxide
Samplex operator	Red phosphorus, potassium chlorate, some of them were using PPE
Box making operator	Scarce exposure to dry matchstick chemicals
Control	No exposure to matchstick chemicals

PPP: Personal protective equipment

Table 7. Effect of tobacco on TCS.

Subjects	*N*	TCS
Control		
Non smokers	22	41.8 ± 22.0
Smokers	26	63.1 ± 19.4
Exposed		
Non smokers	60	73.4 ± 45.4*
Smokers	32	78.7 ± 49.3*

*Difference significant *$P < 0.05$ relative to controls (Student's *t*-test, two-tail)

chemicals in such industrial environments could induce DNA damage [28]. The increased genotoxicity in individuals occupationally exposed to these chemicals might pose an increased risk of cancer.

Our results demonstrated that workers with a long-term chemical exposure in match industry had a slightly higher TCS value (Table 3) indicating that extended duration of exposure might increase DNA damage.

Our results pointed out that mean Age had a non-significant difference ($P < 0.05$) between control and exposed group (Table 4). The effect of age on DNA damage is not exactly defined although some reports described an increase in this parameter with age [29–33]. Similar to our results, some previous studies also showed that age has no significant effect on DNA damage [34–36].

The present study displayed the significance of the nature of the task in the factory as workers involved in handling the matchstick chemicals (directly exposed workers) while not using effective protective measures, showed increased genetic damage. The highest TCS score of sweepers made evident that they were the most high-risk group of workers in all labor categories in our study. This likely reflected this task may involve higher exposures that occur without the use of precautionary measures such as the use of gloves, protective clothing, or respirator mask. The workers lacked the awareness related to chemical handling and self-hygiene and were mostly illiterate. The smallest TCS 26.0 ± 25.0 of the box making operators may be due to less exposure because of dry matchstick chemical coats on match box paper and might use of precautionary measures, that is latex gloves, apron, plastic goggles and respirator mask (Table 5). It was observed that employees with indirect exposure, that is those who were working in the factory but were not involved in the handling of chemicals like security guards, computer operators, sales managers, and office personals showed increased TCS because of inhaling matchstick

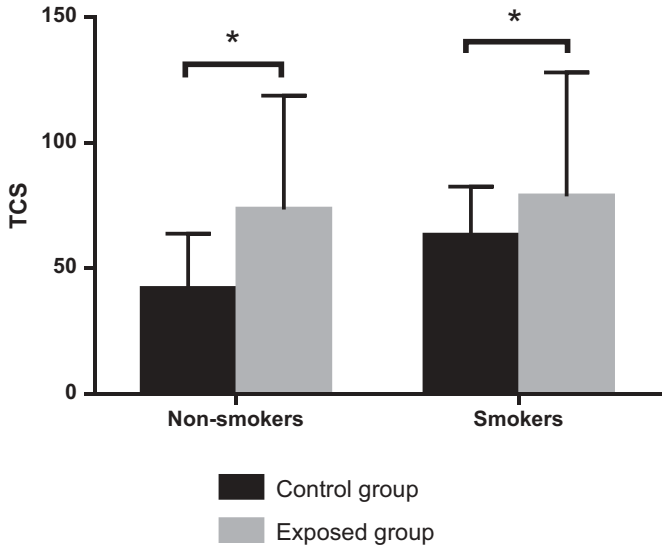

Figure 5. Effect of smoking on TCS. Difference significant *$P < 0.05$ relative to control groups.

chemicals that pervaded the factories without having any protective measures.

Tobacco smoke contains a high number of mutagenic and carcinogenic substances, so smoking is an important variable to consider in biomonitoring studies [37,38]. The present study indicated that smoking increased the level of DNA damage among both matchstick factory workers and controls (Table 7). Among the exposed smokers, the TCS was higher than in exposed non-smokers, but the difference was non-significant. It is possible that the described influence of tobacco use among subjects could be related to the influence of exposure time and their effect may be additive or even synergic. Similar effects of smoking on DNA damage have been noted in a number of previous comet assay studies [34,39–44]. In contrast, some studies have shown that there is no difference regarding DNA damage between smokers and non-smokers [45–47].

A major limitation of the present study is its small sample size. Despite this limitation, significant differences in TCS were identified. The study did evaluate differences in TCS by work area, but attributing this effect to specific exposures is limited due to the inability to assess the matchstick chemicals in the ambient environment. Thus, the effects reflect the impacts of the working environment, but cannot be associated with exposure to any particular agent from this study. For future studies, investigations to better characterize exposure profiles will enhance the utility of the work to identify causes and design appropriate risk management strategies.

Conclusion

Matchstick manufacturing involves exposures to a variety of chemicals, including red phosphorus, neutralizers, carbon black and potassium chlorate. We show in this study that working in these environments in the absence of exposure controls may cause genotoxic effects and increase DNA damage in matchstick workers during occupational exposure, as compared to control subjects as assessed by comet assay. It would be practical to educate the workers who are exposed to matchstick chemicals about the potential hazards of occupational exposure and the importance of using protective measures. Since DNA damage is an important step in events leading from carcinogen exposure to cancer, our study represents an important contribution in evaluating the potential health risk associated with matchstick chemicals exposure. An important outcome of our study is that the management of the matchstick factories where we conducted this work has started implementing safety measures to prevent chemicals exposure to their workers.

Acknowledgments

The authors thank the participants for their contribution to this research, and the Physician at Hospital in Kharkhano Industrial estate Peshawar for their support in conducting this study. We also acknowledge Dr. Jawad Ahmad Director Khyber Medical University Peshawar for providing the facility of florescence microscope.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research work was funded by the University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

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