METANUCLEAR ABNORMALITIES IN FORMALIN-EXPOSED INDIVIDUALS

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ABSTRACT

BACKGROUND
Formalin is a genotoxic agent which causes respiratory illness on mild exposure and cancer on chronic exposure. This study provides an easy and inexpensive way to assess the nuclear abnormalities.

MATERIALS AND METHODS
Fifty individuals with different duration of exposure were included in our study. Oral squamous cells were collected by scraping the wooden spatula. The slides were fixed with methanol glutaraldehyde fixative and stained with Giemsa, May-Grunwald stains. After air drying, 1000 cells were screened for metanuclear abnormalities.

RESULTS
Metanuclear abnormalities were more in the group who had more than 15 years of formalin exposure. The mean value was 66.80. The frequency of metanuclear abnormalities were significant within the groups and between the groups also.

CONCLUSIONS
The genotoxic effect of formalin was confirmed by the increase in the nuclear abnormalities in the formalin-exposed group.

KEYWORDS
Karyolysis, Karyorrhexis, Binucleated Egg, Formalin, Genotoxicity.


BACKGROUND
Formalin is a 40% solution of formaldehyde in water.\(^1\) Industrial workers, professional groups have high exposure (embalmers).\(^2\) Formalin causes cancer.\(^3\) First listed human carcinogen was Formaldehyde CAS No. 50-00-0 as per the Second Annual Report on Carcinogens (1981). Formaldehyde exposure causes head and neck cancers mainly the nasal, lymphoid and oral cancers. An excess of brain cancer mortality was found in all studies of professional group.\(^4\)

Among males, cancers of lung, mouth, oesophagus and stomach are the leading sites across most of the registries.\(^5\) Genetic damage, mutation in critical genes are related to carcinogenesis. Genetic damage evaluation, mutation and genetic instability can be used as a biomarker to assess the level of lesion,\(^6\) karyorrhexis (KR) and binucleated eggs (BN) (Fig. 1). Karyolysis (KL) (Fig. 2) is the nuclear change resulting from genotoxicity.\(^6\)

Aims & Objectives are:
To study the formalin-induced changes in the oral mucosa.
To detect the nuclear abnormalities in the formalin-exposed individuals.

MATERIALS AND METHODS
Study Design- Descriptive study.
Statistical Method- One-Way ANOVA.
SPSS Version 21 was used.
Materials- Methanol, Glacial acetic acid, Giemsa stain and May-Grunwald stain.

Inclusion Criteria
Faculty and staff working in the Department of Anatomy and the first-year students were taken as study sample that consisted of 50 subjects and divided into 5 groups as follows:

Group 1- Less than 1 year of formalin exposure.
Group 2- 1 – 5 years of formalin exposure.
Group 3- 6 – 10 years of formalin exposure.
Group 4- 10 – 15 years of formalin exposure.
Group 5- More than 15 years of formalin exposure.

Exclusion Criteria
Alcoholics, smokers, pre-malignant cases and malignant cases.

After getting the informed consent, the individuals were asked to rinse the mouth and the material was collected from the oral cavity by scraping the buccal mucosa using a clean wooden spatula. Scrapped material was spread on cleaned slides and smeared. After air drying, the slides were kept in the methanol: glacial acetic acid fixative in the proportion 3:1 for 20 minutes. There fixed slides were stained with May-
Grunwald and Giemsa stain. They were observed for Nuclear abnormalities like Karyolysis (KL), Karyorrhexis (KR), Binucleated egg (BN) under bright field Nikon microscope under 10 x 100 magnifications. (Fig 1, 2) Observations were recorded and tabulated. 1000 cells were screened in each person from the slides prepared and the frequency of Metanuclear abnormalities were recorded and the collected data was subjected to one-way ANOVA by using SPSS software version 21.(7)

RESULTS
One-way ANOVA was used to compare the frequency of nuclear abnormalities and the duration of exposure. Table 1 shows increase in the frequency of Metanuclear abnormalities in the group who had more than 15 years of exposure followed by the group who had 10-15 years of exposure. The frequency of metanuclear abnormalities was low in the group that had less than 1 year of exposure. The bar diagram shows that 66.8% of metanuclear abnormalities were more in the group that had more than 15 years of formalin exposure. (Fig 3).

DISCUSSION
Formaldehyde causes nasopharyngeal cancer and leukaemia in humans. (8) The exposure level was more while doing the macroscopic examination of formalin-preserved specimens. (9) Chronic exposure at young age causes delayed health effects, including cancer. In our study, the first-year students were exposed to more than 2 hours in the dissection hall for one-year period of time. So they are more prone to health-related issues,(10) the most important marker of cell death was nuclear abnormalities. Fragmentation of the nucleus in the cytoplasm results in Karyorrhexis (KR) (Fig. 1). Digestion of the chromatin results in a cell with no nucleus which is known as Karyolysis (KL) (Fig. 2). (13)

Claudia et al found that nuclear abnormalities were more in formalin-exposed individuals as stated in our study,(12) relative increase in the nuclear abnormalities found in the outdoor formaldehyde exposure studies were well in correlation with our study which included the indoor formalin-exposed individuals. (13) Metanuclear abnormalities were more in the workers and faculties working in the dissection hall for more than 10 years of exposure. These results were well in correlation with a study conducted by Shekawat S et al. (14) Leon Cleres Penido et al stated that students with a more extensive workload in anatomy laboratory showed increase in karyorrhexis, the same results were seen in our study and hence we need to reduce the duration of formalin exposure in the dissection hall and should advise the students to wear mask to prevent the absorption of formalin in the nasal epithelium. (15) Cellular injury caused by the formalin in the spray painters led to the significant increase in the nuclear abnormalities which was in correlation with our study where formalin-exposed faculties and attenders had increased number of metanuclear abnormalities. (16)

<table>
<thead>
<tr>
<th>Metanuclear Abnormalities</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
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<tbody>
<tr>
<td>Less Than 1</td>
<td>10</td>
<td>9.60</td>
<td>3.806</td>
<td>1.204</td>
<td>6.88</td>
<td>12.32</td>
<td>5</td>
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<tr>
<td>1 to 5</td>
<td>10</td>
<td>6.50</td>
<td>1.581</td>
<td>.500</td>
<td>5.37</td>
<td>7.63</td>
<td>5</td>
</tr>
<tr>
<td>6 to 10</td>
<td>10</td>
<td>21.20</td>
<td>4.492</td>
<td>1.420</td>
<td>17.99</td>
<td>24.41</td>
<td>12</td>
</tr>
<tr>
<td>10 to 15</td>
<td>10</td>
<td>58.70</td>
<td>20.651</td>
<td>6.530</td>
<td>43.93</td>
<td>73.47</td>
<td>28</td>
</tr>
<tr>
<td>Above 15</td>
<td>10</td>
<td>66.80</td>
<td>20.225</td>
<td>6.396</td>
<td>52.33</td>
<td>81.27</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>32.56</td>
<td>28.487</td>
<td>4.029</td>
<td>24.46</td>
<td>40.66</td>
<td>5</td>
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</table>

Table 1. One-way ANOVA (Comparison Between Duration of Exposure to Metanuclear Abnormalities)

<table>
<thead>
<tr>
<th>Metanuclear Abnormalities</th>
<th>Sum of Squares</th>
<th>Degree of Freedom</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
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<td>Between Groups</td>
<td>31910.120</td>
<td>4</td>
<td>7977.530</td>
<td>45.707</td>
<td>0.000</td>
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<tr>
<td>Within Groups</td>
<td>7054.200</td>
<td>45</td>
<td>174.538</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>39764.320</td>
<td>49</td>
<td></td>
<td></td>
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</tbody>
</table>

Figure 1. This Figure shows 1. Karyorrhexis and 2. Shows the Binucleated Egg

Figure 2. This Figure shows the Distorted Nuclear Membrane – Karyolysis (KL)
CONCLUSION
Nuclear abnormalities are more among the formalin-exposed individuals who had more than 10 years of exposure. Nuclear abnormalities may lead to health-related issues and cancer which is in relation with the duration of exposure. Anatomy dissection hall attenders, faculties and students are the more vulnerable groups. To prevent this, the above said groups can wear mask and the exposure time can be minimised. Apart from this metanuclear assay, the other tests like automated micronucleus assay can be done in this large population to find out the lesions at an earlier stage.

REFERENCES