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Original Research Article

FORMALIN INDUCED NUCLEAR ABNORMALITIES IN RELATION WITH DURATION OF EXPOSURE: A STUDY OF FORMALIN EXPOSED ANATOMISTS, ATTENDERS AND STUDENTS

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ABSTRACT

Background: Exposure of Genotoxic agents like formalin causes cancer. The duration of exposure and the quantity of exposure plays an important role in inducing the nuclear abnormalities. The nuclear abnormalities are BN, KR, KL.

Objective: This study provides an easy and inexpensive way to assess the formalin induced nuclear abnormalities in different groups with different duration of exposure.

Materials and methods: Fifty individuals with different duration of exposure were included in our descriptive study. Oral squamous cells were collected with 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 nuclear abnormalities like BN, KR, KL.

Results: Nuclear abnormalities like KR, KL, BN were assessed. KL is more in less than 1-year exposure group.KR is more in long term exposure groups. BN cells are seen 6 – 10 and 10 – 15 years exposure group.

Conclusion: The genotoxic effect of formalin was confirmed by the increase in the nuclear abnormalities especially by the increase in the frequency of KR in tong term exposed group and KL in less than 1-year exposed group.

KEY WORDS: Karyolysis, Karyorrhexis, Binucleated cell, Formalin, Genotoxicity.

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INTRODUCTION

40% solution of formaldehyde in water is formalin.[1] Embalming workers, Industrial workers have high exposure [2] Formalin causes cancer.[3] Formaldehyde CAS No. 50-00-0 is the first listed carcinogen. Formalin exposure causes Head and neck cancers, brain cancer and oral cancers.[4] According to the cancer registries, among males, cancers of lung, mouth, oesophagus and stomach are the leading sites of cancer.[5] carcinogenesis is caused by Mutation and Genetic damage. Genetic damage evaluation, mutation and genetic instability can be used as a biomarker to assess the level of lesion,[5] karyorrhexis (KR) and binucleated eggs (BN) (Fig. 1). Karyolysis (KL) (Fig. 2) is the nuclear change resulting from genotoxicity.[6] Aim is to study the formalin-induced changes in the oral mucosa and to detect the nuclear abnormalities in relation to the duration of exposure.

MATERIALS

Study Design- Descriptive study.

SPSS Version 21 was used.

Materials- Methanol, Glacial acetic acid, Giemsa stain and Maygrunwald stain.

Inclusion Criteria: Faculties 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.

Fig. 1: Karyorrhexis.

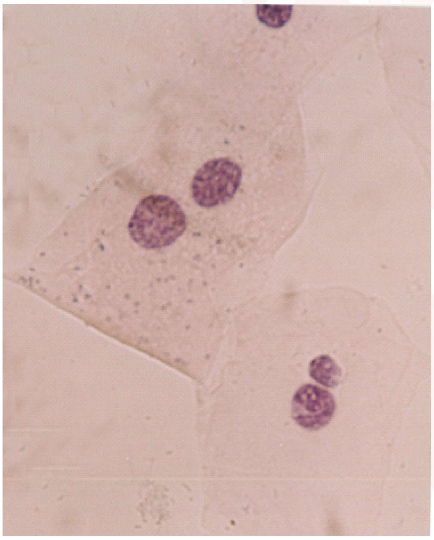


Fig. 2: Binucleated cell(BN).

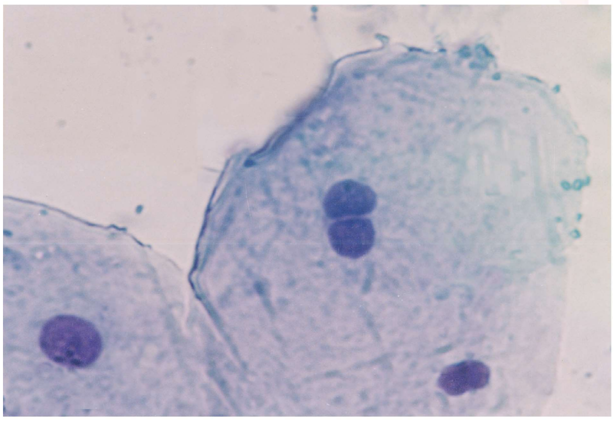
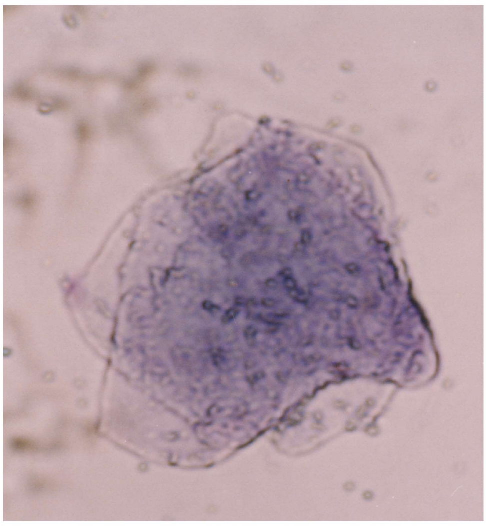


Fig. 3: Karyolysis (KL).



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

Methodology: 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 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, 2,3) 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 AND DISCUSSION

Frequency of metanuclear abnormalities among 5 groups: (Table 1) Among the less than one-year group karyolysis is more,1-5 years exposure group karyorrhexis is more ,6-10 years and 10 – 15 years exposure group binucleated eggs are more in number. More than 15 years exposure group karyorrhexis is more.

Comparison between the years of exposure and metanuclear abnormalities like KR, KL, BN are highly significant. (Table 2)

Dependent variable in BN group (Table 3): Less than 1-year group when compared to other groups the p value is highly significant with all groups. Less than 1 year was not significant when it Was compared with the 1-5 group and the 1-5 yrs. group was not significant with the less than 1 group vice versa. 6-10,10-15 and >15yrs group was highly significant when it is compared with all other groups.

Dependant variable in KR group (Table 3): < 1-year group was significant when it was compared with 10 – 15 years and > 15 years group. 6- 10years group is significant when it is compared with > 15 years group. 10 – 15 years group and > 15 years group were significant when it was compared with all other groups.

Dependant variable in KL group (Table 3): < 1year group was significant when it was compared with 10 – 15 years, > 15 years group. 1-5 year ,6 – 10year groups were significant when compared with 10 – 15 and > 15 group. 10 – 15 and > 15 years were significant when compared with 10 – 15 and > 15 group.

Table 1: Frequency of metanuclear abnormalities in different Groups.

	BN	KR	KL
Less than 1 year	1.8	3.6	4.2
1-5 years	1	3.9	1.6
5 – 10 years	11.7	4.5	5
10 – 15 years	24.6	17.5	16.6
More than 15 years	18.3	29.4	19.1

Table 2: Significance of BN cells among different groups of exposure.

		N	Mean	Std. Deviation	P value
BN	less than one year	10	1.8	1.8	<0.0001
	1 - 5 yrs	10	1	1.1	
	6 - 10 yrs	10	11.7	4.2	
	10 - 15 yrs	10	24.6	7	
	more than 15 years	10	18.3	4.6	
	Total	50	11.5	10.2	
KR	less than one year	10	3.6	2.2	<0.0001
	1 - 5 yrs	10	3.9	1.1	
	6 - 10 yrs	10	4.5	3.9	
	10 - 15 yrs	10	17.5	7.3	
	more than 15 years	10	29.4	20.2	
	Total	50	11.8	14	
KL	less than one year	10	4.2	3	<0.0001
	1 - 5 yrs	10	1.6	0.5	
	6 - 10 yrs	10	5	2.1	
	10 - 15 yrs	10	16.6	10.8	
	more than 15 years	10	19.1	7.2	
	Total	50	9.3	9.2	

Table 3: Dependent variable and duration of exposure of each metanuclear abnormalities.

Dependent Variable			Mean Difference (I-J)	Sig.
BN	less than one year	1 - 5 yrs	0.8	1
		6 - 10 yrs	-9.9	<0.0001
		10 - 15 yrs	-22.8	<0.0001
		more than 15 years	-16.5	<0.0001
	1 - 5 yrs	less than one year	-0.8	1
		6 - 10 yrs	-10.7	<0.0001
		10 - 15 yrs	-23.6	<0.0001
		more than 15 years	-17.3	<0.0001
	6 - 10 yrs	less than one year	9.9	<0.0001
		1 - 5 yrs	10.7	<0.0001
		10 - 15 yrs	-12.9	<0.0001
		more than 15 years	-6.6	0.013
	10 - 15 yrs	less than one year	22.8	<0.0001
		1 - 5 yrs	23.6	<0.0001
		6 - 10 yrs	12.9	<0.0001
		more than 15 years	6.3	0.02
	more than 15 years	less than one year	16.5	<0.0001
		1 - 5 yrs	17.3	<0.0001
		6 - 10 yrs	6.6	0.013
		10 - 15 yrs	-6.3	0.02
KR	less than one year	1 - 5 yrs	-0.3	1
		6 - 10 yrs	-0.9	1
		10 - 15 yrs	-13.9	0.028
		more than 15 years	-25.8	<0.0001
	1 - 5 yrs	less than one year	0.3	1
		6 - 10 yrs	-0.6	1
		10 - 15 yrs	-13.6	0.034
		more than 15 years	-25.5	<0.0001
	6 - 10 yrs	less than one year	0.9	1
		1 - 5 yrs	0.6	1
		10 - 15 yrs	-13	0.05
		more than 15 years	-24.9	<0.0001
	10 - 15 yrs	less than one year	13.9	0.028
		1 - 5 yrs	13.6	0.034
		6 - 10 yrs	13	0.05
		more than 15 years	-11.9	0.096
	more than 15 years	less than one year	25.8	<0.0001
		1 - 5 yrs	25.5	<0.0001
		6 - 10 yrs	24.9	<0.0001
		10 - 15 yrs	11.9	0.096
KL	less than one year	1 - 5 yrs	2.6	1
		6 - 10 yrs	-0.8	1
		10 - 15 yrs	-12.4	<0.0001
		more than 15 years	-14.9	<0.0001
	1 - 5 yrs	less than one year	-2.6	1
		6 - 10 yrs	-3.4	1
		10 - 15 yrs	-15	<0.0001
		more than 15 years	-17.5	<0.0001
	6 - 10 yrs	less than one year	0.8	1
		1 - 5 yrs	3.4	1
		10 - 15 yrs	-11.6	0.001
		more than 15 years	-14.1	<0.0001
	10 - 15 yrs	less than one year	12.4	<0.0001
		1 - 5 yrs	15	<0.0001
		6 - 10 yrs	11.6	0.001
		more than 15 years	-2.5	1
	more than 15 years	less than one year	14.9	<0.0001
		1 - 5 yrs	17.5	<0.0001
		6 - 10 yrs	14.1	<0.0001
		10 - 15 yrs	2.5	1

DISCUSSION

In humans, Formaldehyde causes nasopharyngeal cancer [8]. The exposure level was more while handling the formalin-preserved specimens[9]. In our study, the first-year students were exposed to FA for more than 2 hours in the dissection hall for one-year. 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) [11]. Binucleation occurs at a much higher rate in cancer cells [12] (fig 2). Digestion of the chromatin results in a cell with no nucleus which is known as Karyolysis (KL)[11] (Fig.3). Total number of binucleated cells found manually by Ramadhan D was 64 as seen in our study in the 6-10 and 10 – 15 years exposure group [13] In our study the nuclear abnormalities were more in formalin-exposed individuals as stated by Mergener et al, [14] relative increase in the nuclear abnormalities found in formaldehyde exposure studies were well in correlation with our study [15] Metanuclear abnormalities were more in the workers 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. [16] 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 [17].

Cellular injury caused by the formalin led to the significant increase in the nuclear abnormalities in the spray painters which was in correlation with our study where formalin-exposed individuals had increased number of metanuclear abnormalities [18]. As per Oliver S et al the cytogenic effects of FA exposed subjects were very unlikely to occur in blood cultures of FA exposed subjects. This study results contradicted our results [19]. According to our results more than 5 years to 15 years of exposure to formalin induces the formation of BN .BN are seen more in cancerous conditions [12]. So, the people mainly the anatomy dissection hall attenders and faculties who spend longer time with the specimens are to be screened for BN cells and the exposure can be minimised if the count is more than 4. There were no studies regarding the individual metanuclear abnormalities like KR, KL, BN frequency in relation with the duration of exposure. Since our study clearly showed KL is more in the group which had less than 1-year exposure so mainly the 1st year students can be screened only for karyolysis no need to screen for KR and BN. Since KR is more in Group with more than 15 years of exposure, pathology technicians and dissection hall staffs can be screened for KR. It is difficult to evaluate the complete metanuclear abnormalities and it is time consuming too. In accordance with our detailed study results, only one assay can be done in the particular exposure group. Since we found the dependent variable for each cell, we should not waste time by doing BN assay for less than 1-year exposure group.

CONCLUSION

Metanuclear abnormalities are more among the formalin-exposed individuals. Nuclear abnormalities can cause cancer. The duration of exposure plays an important role in carcinogenesis. Anatomy dissection hall attenders, faculties, Pathology technicians and students are the groups to be screened. Nuclear abnormalities vary with the duration of exposure. To prevent this, the exposure time can be minimised and according to the years of exposure they should be screened for the abnormalities. Manual method is time consuming so automated metanuclear assay can be done in large population to find out the lesions at an earlier stage.

ABBREVIATIONS

BN - Binucleated cell

KL - Karyolysis

KR – Karyorrhexis

Conflicts of Interests: None

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