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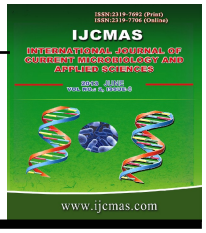

Original Research Article

Nasal floral changes among medical students on exposure to formaldehyde

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Keywords

Formaldehyde; Nasal flora; Anatomy dissection theatre; Bacterial load.

A B S T R A C T

Formaldehyde is a colourless inflammable gas used as one of the commonest chemical in the anatomy dissection hall for the preservation of the cadaver. This study was carried to evaluate changes in the nasal flora following the exposure of formaldehyde among medical students at Chennai Medical College Hospital and Research Centre, Trichy. In this study, a total of 45 students and ten healthy controls who were not exposed to formaldehyde were enrolled. Organisms such as *Klebsiella* spp. (48.88%), *CoNS (44.21%)*, *Pseudomonas* spp. (40%), *Micrococcus* (16.67%), *Diphtheroids* (14.78%), *Staphylococcus aureus (*14.31%), *Providencia* (8.88%), were isolated in the nasal flora of the medical students. There is no significant change in the nasal flora was observed before and after exposure to formaldehyde while the load of the organism were found to be reduced after exposure to formaldehyde. Further studies are needed to implement the standard protocol to be adopted for handling the formalin exposure procedures.

Introduction

Formaldehyde is a colourless, flammable, gas that is soluble in water. It is one of the most important chemicals used in the anatomy dissection hall to preserve the cadaver. It has been purported to be used as early as 1899 for cadaver embalming. It is also used for sterilization purposes like fumigation of operation theatres, wards etc.

It functions as a fixative and anti-microbial agent due to its ability to cross link DNA, RNA and protein. (Alli, 2010) The primary effects of acute exposure to formalin are mucous membrane irritation and allergic sensitization of the skin and lung, and the same have been documented. Formaldehyde had been shown to have cidal effects on microbes (Alli, 2010).

Hence exposure to formaldehyde reduces the throat and nasal flora in human. Anatomy students at medical colleges, through several studies have shown significant negative health effects from the dissection of formaldehyde embalmed cadavers, as these students are exposed to 5ppm-1ppm formaldehyde for 2 hours everyday throughout the year (Bedino, 2004). This causes reversible and significant symptomology, manifested as major health problems as time progresses.

There are numerous research articles pointing to the hazardous effects of formaldehyde and organic solvents on pregnancy including birth defects, foetal abnormalities, spontaneous abortions, short term miscarriage and difficulties. Mutagenic effects following chronic exposure have been demonstrated in vitro and experimental animals have shown carcinogenic effects (Chia *et al*., 1992). Several occupational studies showed an increased risk of nasopharyngeal and sinonasal cancer in workers exposed to high concentrations of formaldehyde (IARC 1995; Environment Canada, Health Canada, 2001).

In a healthy human, the internal tissues like blood, brain, muscle are normally free of organisms. However the surface tissues like skin and mucous membranes are constantly in contact with environmental organisms become readily colonized by various microbial species. The most predominant normal bacterial flora in the nasal cavity is the *Staphylococcus* species.

As upper respiratory tract normal flora plays an important role in keeping at bay the professional pathogens and exposure to formaldehyde has been shown to have sterilizing effect on microbes, we planned to study the immediate changes of the nasal flora among the first year medical students who are exposed to formalin for at least 2 hours every day throughout the year.

Materials and Methods

A prospective observational study was carried out (from June to August 2011) in the medical college after getting informed consent from participants and an approval from Institutional Ethics Committee.

After explaining the study methodology, nasal swabs were collected from 45 medical students before and after exposure to formalin for 2 hrs. For Controls, samples were collected from 10 other individuals who were working in administration block of college, not exposed to formalin. The concentration of formalin used in anatomy dissection hall in the institution was 10%. The participants and controls who were free from acute or chronic rhinitis, sino-bronchial syndrome, disorders related to airways, non-smokers, not used snuff or nasal drops for any other purpose were enrolled in this study . All the participants were explained not to meddle their nostrils with fingers for six hours prior to the study and during next two hours or till the collection of second sample.

Sample Collection

On every Monday, a set of six students were subjected to study. Two sterile saline moistened nasal swabs were collected from the left and right nostril of students before going to the anatomy theatre (Sample 1) and also at the end of two hour after exposure (Sample 2) two nasal swabs were taken from the same students. Each swab was labelled accordingly. Totally 45 students were covered in a period of two months. A total of ten samples were also collected from the control group almost at

the same time and two hours interval and marked as C Sample 1 and C sample 2 respectively. The swabs were transported immediately to the microbiology laboratory without any delay in transport media and the same were processed.

Microbial flora of anatomy dissection hall

Nutrient agar and Sabroud's dextrose agar plates were exposed in the Anatomy dissection theatre for half an hour to analyze the microbial flora of the same on every Monday at about1 pm after the students left the dissection theatre.

Processing of samples:

One set of the nasal swabs were inoculated into Brain Heart Infusion broth (BHIB) and incubated overnight. The next day it was subcultured on nutrient agar, blood agar, MacConkey agar media and incubated aerobically at 37ºC. The plates were examined after overnight incubation and the organisms were identified by gram staining and standard biochemical tests. The gram positive cocci were identified by catalase test, oxidation/fermentation test, coagulase test and mannitol fermentation test. *Staphylococcus aureus* isolates were tested for methicillin resistance using oxacillin screening agar plate. Similarly gram negative bacilli were identified by catalase test, oxidase test and motility by hanging drop, IMViC tests and sugar fermentation using triple sugar iron agar medium. Antibiotic sensitivity was performed on Muller- Hinton agar using Kirby-Bauer disc diffusion technique.

Bacterial count

Another set of sample were placed in one ml of Ringer's solution and immediately processed for bacterial count. The bacterial count (surface viable count) of organisms was done using Miles and Mistra method. Chocolate agar plate was dried and the plate was divided in to 8 segments represented as 10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} and 10^{-8}. Tenfold dilutions of the sample were carried out in 1 ml of Ringer's solution to cover dilution range from 10^{-1} to 10^{-8}. From each dilution of the sample, 10 micro litre was taken and dropped on to labelled segment of chocolate agar plate and the drop was allowed to be absorbed by the agar and incubated in 5% CO_2 incubator at 37^{o}C for overnight. The number of colonies were counted and recorded in colony forming units (CFU).

Data Analysis

Data were entered in computer and analysed using SPSS statistical package.

Result and Discussion

Nasal bacterial flora of the medical students before and after exposure to formalin along with their antibiotic sensitivity pattern in relation to gram positive and gram negative organisms are depicted in table 1 and 2 respectively. Similar data for the control with reference to C sample 1 and 2 are given in table 4 and 5 respectively. One of the *Staphylococcus aureus* strain isolated from a student was Methicillin resistant *Staphylococcus aureus* (MRSA).

Single type of organism was isolated more from control samples and two types of organisms were more from medical

Table.1 Gram positive bacteria among medical students

Name of Bacteria	**Sample1 n=90**	**Sample 2 n=90**	**sensitivity pattern (%)**								
			AMP	AC	CX	COT	E	DO	CIP	VA	AK
CoNS	41 (45.05)	36 (43.37)	97.4	92.2	97.4%	84.4	93.5%	98.7	96.1	100	87.0
Micrococci	15 (16.48)	14 (16.86)	-	-	-	-	-	-	-	-	-
S. aureus	14 (15.38)	11 (13.25)	100	*100*	*96*	*76*	*84*	*96*	*96*	*100*	*84*
Diphtheroids	20 (23.07)	22 (26.50)	-	-	-	-	-	-	-	-	-

Sample1 - before exposure of Formaldehyde Sample 2 - after exposureof Formaldehyde; AMP – Ampicillin AC - Amoxyclav, CX-Cloxacillin, CO-CotrimoxazoleE - Erythromycin, DO-Doxycyclin CIP –Ciprofloxacin VA - Vancomycin, AK- Amikacin

Figure.1 Change in microbial flora after exposure to formalin

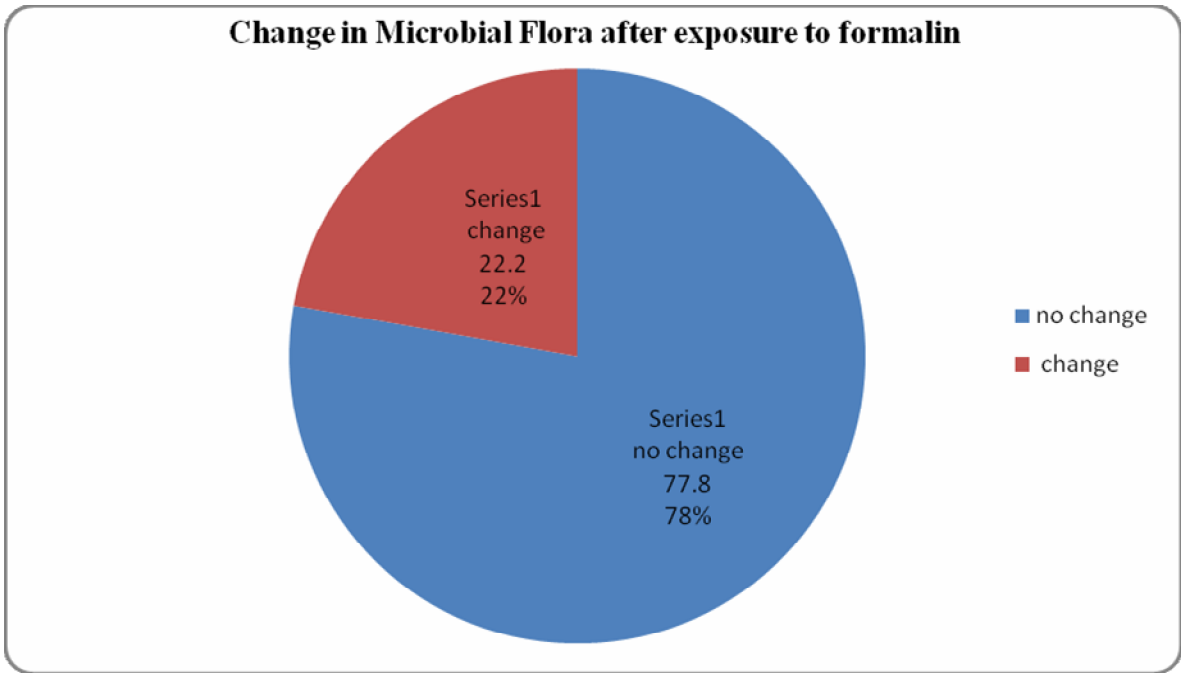

Table.2 Gram negative isolates among medical students and their antimicrobial sensitivity pattern

Name of Bacteria	Sample 1 n=90	Sample 2 n=90	Total	Sensitivity pattern								
				COT	AMP	AC	GEN	AK	DO	CIP	CTR	CAZ
Klebsiella pneumoniae	20 (44.44%)	20 (44.44%)	40	100%	95%	90%	90%	95%	100%	100%	85%	95%
Klebsiella oxytoca	2 (4.44%)	2 (4.44%)	4	100%	100%	100%	100%	100%	100%	100%	100%	100%
Pseudomonas spp.	18 (40%)	18 (40%)	36	97.2%	72.2%	100%	100%	36.1%	75%	47.2%	91.6%	97.2%
Providencia	4 (8.88%)	4 (8.88%)	8	100%	100%	100%	100%	100%	100%	100%	100%	100%
Acinetobacter	1 (2.22%)	1 (2.22%)	2	100%	100%	100%	100%	100%	100%	100%	100%	100%

Sample1 - before exposure of formalin; Sample2 - after exposure of formalin; COT - Co-trimoxazole, AMP – Ampicilin, AC –Amoxyclav, GEN-Gentamicin, AK- Amikacin, DO-Doxycyclin, CIP –Ciprofloxacin, CTR-Ceftriaxone,CAZ-Ceftazidime .

students (Table 3) and the difference was statistically significant ($p<0.01$).Also gram negative isolates were more from medical students than control and the difference was statistically significant ($p<0.01$).Sizeable number of isolates of both gram positive and gram negative were sensitive to most of the antibiotics used in clinical practice.

Only ten students (22.2%) showed changes in the nasal flora after exposure to formalin which is shown in Fig 1. Comparative analysis of bacterial isolates before and after formalin exposure among students did not reveal any significant variation. Similarly no variation was noticed among the isolates obtained from sample 1 and sample 2 of controls.

The bacterial load of nasal flora of the students before and after exposure to formalin was in the range of 6×10^4 to 2×10^5 and 5×10^4 to 1×10^5 respectively. There was a significant ($p<0.01$) reduction in the bacterial load after exposure to formalin. The bacterial load of C sample 1 and 2 of control was in the range of 5×10^4 to 2×10^5 on both occasions without any difference.

There was no bacterial growth in the plates exposed in the anatomy dissection theatre. This study was designed to determine to find out the nasal flora before and after exposure to formalin among first year medical students. In this study, *Klebsiella* spp. (48.88%), *CoNS* (44.21%), *Pseudomonas* spps(40%), Micrococcus(16.67%), Diphtheroids (14.78%), *Staphylococcus aureus (*14.31%), Providencia (8.88%), were isolated in the nasal flora of the medical students. Thus *Klebsiella* spp was dominant followed by *CoNS* and *Pseudomonas* spp. This is in accordance with Alli *et al*., (2010) who observed that Klebsiellaspp as the dominant bacteria in the nasal swab of 20 human subjects studied.

It was observed that there is no significant change in the bacterial isolates and significant reduction in the growth before and after exposure to formalin. Antibiotic sensitivity test showed that all gram positive and gram negative bacteria were sensitive to the commonly used antibiotics. MRSA was isolated from only one student.

The microbial floras are in ecological balance protecting the host from pathogens. Different mechanisms have been proposed to explain the protective effects which include the production of substances such as bacteriocins, organic acids, hydrogen peroxide and steric hindrance competition for nutrients. When there is reduction in the number of these microbial floras, all the above mechanisms can be disrupted and as such foreign pathogens will be able to thrive and cause infections. This may be the probable reason for the occurrence of gram negative bacteria such as *Klebsiella, Providencia* and *Pseudomonas* which were more among medical students than controls.

According to Alli *et al*., (2010) the reduction in the normal flora of humans were found to be statistically significant while reduction in the normal flora of rabbits were significant when compared between controls and those exposed to 10% formaldehyde but were not significant between the controls and those exposed to 100% formaldehyde. This could be due to formaldehyde being more

Table.3 Nasal flora of the medical students and the controls

Organisms Obtained	Medical students				Control			
	Sample-1		Sample-2		C - Sample - 1		C – Sample - 2	
	Right (n=45)	Left (n=45)	Right (n=45)	Left (n=45)	Right (n=10)	Left (n=10)	Right (n=10)	Left (n=10)
Single type	18 (40%)	26 57.7%)	23 51.1%)	29 (64.4%)	8 (80%)	8 (80%)	8 (80%)	8 (80%)
Two types	27 (60%)	19 (42.2%)	22 (48.8%)	16 (35.5%)	2 (20%)	2 (20%)	2 (20%)	2 (20%)

Sample 1 - before exposure of formalin Sample 2 - after exposure of formalin; C Sample 1-nasal swab during the first sample of the medical student; C sample 2- 2 hours after sample 1

effective as a disinfectant at lower concentration than at higher concentration. Apart from the changes in the nasal flora, formalin is toxic to upper respiratory tract and causes irritation of the eyes and nose, respiratory impairment, difficulty in breathing and so on (Yodaiken, 1981).

In our study the bacterial isolates did not alter after exposure to formalin but bacterial load of nasal flora came down significantly after exposure to formalin. This indicates that acute exposure to formalin has an effect on the nasal bacterial flora. However more such studies are suggested to confirm or refute this observation.

Kikuta *et al*., (2010) suggested that that the formaldehyde level could be reduced and it is mandatory to provide better air circulation by introducing new air devices, to meet the specifications of a well designed and constructed anatomy dissection hall.

Hence further studies are needed to study and compare the nasal flora of the medical students and control people who exposed to formalin regularly to implement the standard protocol to be adopted for handling the formalin exposure procedures (i.e people working in anatomy dissection hall, operation theatre pathology laboratory etc).

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