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Original Article

Effect of Formaldehyde on the Upper Respiratory Tract Normal Flora of Humans and Rabbits

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ABSTRACT

Background: Formaldehyde is a chemical that is used to fix a tissue after death or removal from the body to prevent autolysis and putrefaction. Exposure to formaldehyde can occur as a result of occupation.

Objective: To determine the effect of the formaldehyde on the throat and nasal flora of upper respiratory tract of rabbits and humans were examined in this study.

Materials and Methods: This study was conducted in the College of Health Sciences (Mercyland campus) and Teaching Hospital of Ladoke Akintola University of Technology, Osogbo. Ten rabbits and 25 human subjects were used. Throat and nasal swabs were taken from these rabbits exposed to different concentrations of formaldehyde and human subjects exposed to formaldehyde as a result of occupational exposure and non exposed individuals. Bacterial count was done using Miles and Mistral method. Microbial culture was done using the traditional cultural techniques.

Results: Culture yielded growth of different species of microorganisms, majority of which were bacterial species. Bacterial counts showed reductions both in normal flora of upper respiratory tract of rabbits and humans. The reduction in the normal flora of humans was found to be statistically significant. While reduction in the normal flora of rabbits was significant when compared between controls and those exposed to 10% formaldehyde, but were not significant between controls and those exposed to 100% formaldehyde.

Conclusion: This study concluded that there was significant reduction in the normal flora of humans and rabbits exposed to formaldehyde compared to non-exposed humans and rabbits.

Key words: Formalin, formaldehyde, normal flora, Upper respiratory tract

Formalin is one of the most important chemical used in histopathology/ histology laboratory for fixing tissues before processing for histological examination. It is also accepted method of sterilization where microbiological cleanliness is required. Fumigation is usually carried out by mixing permanganate and an excess formalin (40% formaldehyde) in a suitable container, sufficient heat is generated by oxidation of part of the formaldehyde (HCHO) with permanganate to vaporize the remaining formaldehyde and water[1-3]. Formaldehyde owes its functions as fixative and microbicidal agent due to its ability to crosslink DNA, RNA, and protein [4,5].

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Exposure of living cells to formaldehyde results in covalent linkage with exposed amino and imino groups (notably in lysine and arginine side chains). This reaction forms a Schiff's base that can participate in a second linkage, creating methylene bridges between amino acids that were in close proximity in the native protein. The effect of this on fixed tissue is the maintenance of molecular relationships to reflect living cells. In histopathology laboratory, HCHO can either be used as a single chemical compound as simple fixative or as part of the chemical compound of compound fixative. Because of the proven carcinogenic nature of the HCHO and the nasal irritation, the use of fume cupboard/ventilation system has been put in place to curtail the spread of the vapour, thereby preventing unnecessary exposure to the formalin. Even with that, there are some laboratories that are still sloppy in terms of exposure of laboratory workers to formalin.

Animal studies have shown that animals stand a risk of developing nasal carcinoma as a result of exposure to HCHO[6]. The study carried out among industrial workers exposed to formaldehyde could not give strong association between cancer and exposure to this chemical[7]. Upper respiratory tract normal flora plays important role in putting at bay professional pathogen. Formaldehyde has been shown to have sterilizing effect on microbes [1]. The consequent of this to humans exposed to formaldehyde will be to have lesser bacterial loads than the unexposed individuals. It was on this premise, that this study was undertaken. The aim of the project was to determine the effect of HCHO on the normal flora of upper respiratory tracts of humans and rabbits.

Materials and Methods

Study design. The study was conducted at College of Health Sciences (Mercyland campus) and Teaching Hospital of Ladoke Akintola University of Technology Osogbo, Nigeria. Ethical approval for the use of laboratory animals was given by ethical committee of the Department of Biomedical Sciences, Ladoke Akintola University of Technology, Osogbo, Nigeria. Ten rabbits (males and females) and ten people working in histopathology laboratory and mortuary were used as tests and 15 people who were not exposed to formaldehyde at all as controls. The duration of experiment was eight weeks. All the rabbits were adults of average weight of about 1.5 - 2.0 kg and well fed. The people used for this project were normal and healthy adults. The rabbits were grouped into three - those exposed to concentrated formalin i.e. 40% formaldehyde aqueous solution, those exposed to 10% formalin, and those that are not exposed at all to the chemical (negative control). The groupings were 4 rabbits for 10% formalin, 4 rabbits for concentrated formalin and 2 rabbits as control.

All the three groups of rabbits were placed in three different rooms in cages. The rabbits were continuously exposed to the required concentration of formalin all through the period of experiment with constant renewal in the morning and evening. Exposure was done by soaking cotton wool in formalin, put in bowls then placed in their various rooms. Replacement was done morning and evening in order to maintain the concentration of formalin.

The first set of samples from rabbits was collected before any of them was exposed to formalin in order to get the baseline normal flora and count.

Specimen collection. With the technical assistance of an Animal Technologist, a moistened sterile swab stick each was inserted into the nasal cavity and throat of each animal to collect sample and each swab stick was labelled accordingly. Two samples were collected from each site - nasal cavity and throat swabs from each animal using sterile swab stick moistened with sterile normal saline, one for bacterial count and the other for bacterial identification. The sample collected was placed in 1 ml of Ringer's solution and immediately processed for bacterial count as described below.

Bacterial count: Bacterial count (surface viable count) of organisms was done using Miles and Mistra method[8]. Chocolate agar plates were dried in the oven to prevent fluid from running over the surface of the plate. The plate divided into 8 segments represented as 10-1-10-2, 10-3, 10-4, 10-5, 10-6, 10-7, and 10-8. Ten fold dilutions of the sample were carried out in 1 ml Ringer's solution to cover dilution range from 10-1 to 10-8. From each dilution of the sample, 20 µl was taken and dropped on to labelled segment of chocolate agar plate. The drop was allowed to be absorbed by the agar and incubated in 5% C02 incubator at 37°C overnight. This was done in triplicates. The number of colonies was counted after overnight incubation.

Cultural technique

Bacterial culture was done using the traditional systematic bacteriological techniques. The culture media used for this experiment were blood agar, chocolate agar, MacConkey agar, and Saboraud agar. Swabs were inoculated on various agar plates and incubated at appropriate temperatures. The

colonial morphologies of various isolates were studied after overnight incubation at 37°C for bacteria and 35oC for 48 hours for fungi on Saboraud agar[9]. For further identification, the colonies were Gram stained and after which further biochemical tests were done to identify each isolate.

Human experiment

With the help of a medical doctor, samples were collected (both nasal and throat swabs) from people used for this study (both tests and controls) after informed consent was taken from the individuals. The bacterial flora and load were determined in triplicates as described above.

Results

Exposure to formaldehyde reduces the throat and nasal flora of upper respiratory tract of rabbits.

In this study, 10 adult rabbits were used. All the samples taken from these rabbits yielded growth of different species of microorganisms, majority of which were bacterial species. The culture from animal samples was obtained after incubating chocolate agar plates at 37oC both aerobically and in the presence of 5% CO_2 overnight. All the organisms isolated were aerobes or facultative anaerobes. With Saboraud agar incubated for 48 h, *Candida spp* were isolated. It was observed that the same sets of organisms were isolated before, after 4 and 8 weeks of exposure to formaldehyde, but there were reductions in number. It was discovered that the predominating organism in the nasal cavity of rabbits was *Staphylococcus species* while *Klebsiella species* predominated in the throat. In addition to this, *Candida albican* was isolated from only the throat of rabbits. Majority of the *Staphylococcus species* isolated from the nasal cavity were coagulase negative *staphylococci*. *Proteus spp* were isolated both from the throat and the nasal cavity. *Escherichia coli* were also isolated from the throat of rabbits.

The results of the bacterial counts done using Miles and Mistral method showed that the load of throat flora was more than those of the nasal flora in all the rabbits sampled. It was also observed that there were reductions in the number of throat and nasal flora after the first 4 weeks of exposure of the rabbits to formalin (Fig. 1 and 2) further reduction after 8 weeks of exposure.

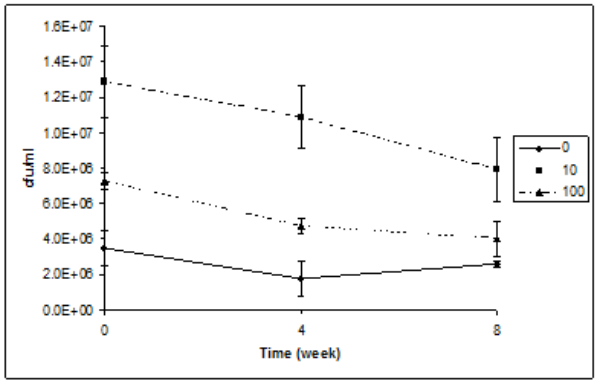

Fig 1. Effect of formaldehyde on the nasal flora of rabbits. Rabbits were exposed to different concentrations of formalin. Total viable bacterial count was determined as described in the materials and methods. The error bars represent the standard error of the bacterial count.

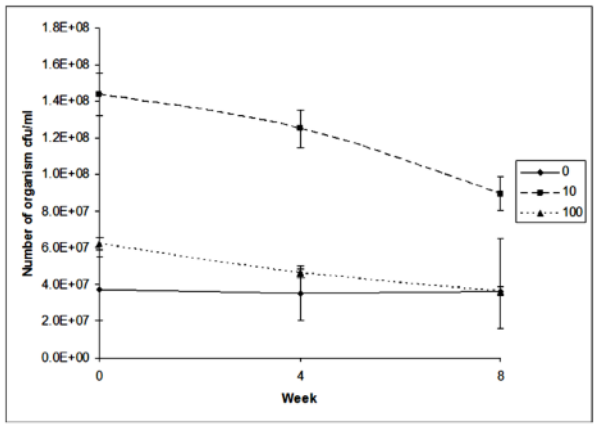

Fig 2. Effect of formaldehyde on the throat flora of rabbits. Rabbits were exposed to different concentrations of formalin. Total viable bacterial count was determined as described in the materials and methods. The error bars represent the standard error of the bacterial count.

The total microbial load of the throat flora ranged from 2 × 107 – 4 × 108 and 2.5 × 106 – 9.5 × 106 for the nasal flora on exposure to different concentrations of formalin (Fig. 2). When the effect of formalin on the nasal flora was compared between unexposed rabbits (controls) and rabbits exposed to 100%, the difference was found not to be statistical significant (T-test $p > 0.05$) while comparison between controls and those exposed to 10%

formalin gave statistical significant difference (T-test $p < 0.05$). In addition, when the effect of formalin was compared between those rabbits exposed to 10% formalin and those exposed to 100% formalin, the difference was found to be statistical significant (T-test $p < 0.05$).

Exposure to formaldehyde reduces the throat and nasal flora in humans. Formaldehyde had been shown to have cidal effect on microbes[1]. In this study, 20 human subjects were used. All the samples taken from the humans both control and test subjects (individuals working in Histopathology laboratory) yielded growth of different species of microorganisms, majority of which were bacterial species. The bacterial species isolated from nasal swabs were Staphylococcus species (of which there was isolation of *Staphylococcus aureus*) and coliforms (dominated by *Klebsiella spp* and *E. coli*) in both the human subjects exposed and unexposed to formaldehyde. It was observed in human subjects (both test and control) that *Streptococcus spp* and *Candida albicans* were isolated from throat in addition to the aforementioned organisms isolated from nasal swabs. In contrast to animals (rabbits), not all organisms isolated in rabbits were represented in humans suggesting differences in the normal flora as a result of host specificity. It was observed that the predominating organisms in the throat of all humans sampled for this study (both test and control) were *Streptococcus spp*. Also, *Candida albican* was isolated only in the throat samples. *Klebsiella spp* were isolated both in the throat and nasal cavity of humans sampled for this project.

The results of the total bacterial counts done on human subjects showed that the bacterial load of throat flora was more than those of nasal flora in all the humans sampled. The microbial loads ranged from 8 × 106 – 1 × 107 for throat and 1 × 106 to 2.5 × 106 for nasal swabs from human subjects (Fig. 3). The microbial counts ranged from 1 × 107 to 9 × 107 for throat and 9 × 106 – 1 × 107 for nasal swabs from the human controls (Fig. 3).

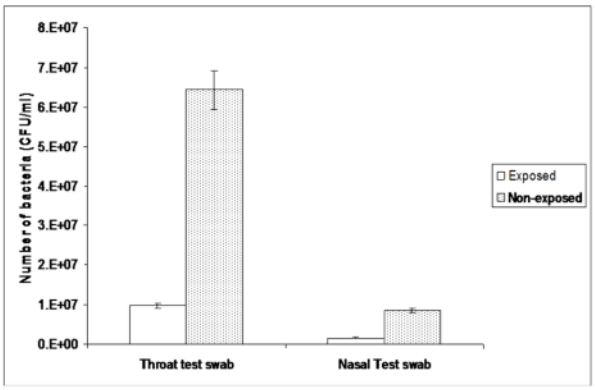

Fig 3. Effect of formaldehyde on the nasal and throat flora of humans exposed to formaldehyde. Total viable bacterial count was determined as described in the materials and methods. The error bars represent the standard error of the bacterial count.

When the effect of formaldehyde on the throat flora of humans was compared between the test and control subjects, the difference was found to be statistically significant (T-test $p < 0.05$). Also, the effect on the nasal flora was found to be significantly different (T-test $p < 0.05$) between the test and control subjects.

Discussion and Conclusion

This study was designed to determine the effect of formaldehyde on the normal flora of upper respiratory tract of rabbits and humans regularly exposed to formalin (aqueous form of formaldehyde) as a result of their occupations. Soon after birth, the open cavities of man and animals are colonized by a wide variety of microorganisms. They become established in the oropharynx, intestinal, and vaginal tracts, constituting the indigenous microflora. These normal indigenous flora can act competitively with foreign pathogens, thus inhibiting the colonization of these pathogens and consequently preventing infections[10]. However, this is true only for certain components of the indigenous flora and certain pathogens.

In this study, the baseline flora of throat and nasal cavity of rabbits were first carried out so as to be able to see the difference in number of normal flora after exposure to formaldehyde.

The roles play by normal flora in health cannot be overemphasized. These microbial flora 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 as bacteriocins[11], organic and hydrogen peroxide[12], steric hinderance[13], competition for nutrients[14]. When there is reduction in the number of these microbial flora, all the above mechanisms can be disrupted and as such, foreign pathogens will be able to thrive and cause infections. Infections of the upper respiratory tract include laryngitis, pharyngitis (sore throat), tonsillitis, epiglotitis, and lower respiratory tract infections. The importance of this has been demonstrated in humans' gastrointestinal tract where antibiotic associated diarrhoea due to *Clostidium difficile*, in this situation the antibiotic kills majority of the normal flora thereby selecting for toxin producing *C. difficile* which later leads to toxin production with the resultant effect on the individual having diarrhoea[15,16].

It was observed in this study that there were reductions seen in the throat and nasal flora of all the rabbits exposed to formalin and humans who were exposed to formalin on regular basis as a result of their occupation. This reduction occurred probably as a result of the fact that formaldehyde is known to be an effective disinfectant that kills microorganisms such as bacteria, viruses, fungi, and parasites at relatively high concentrations[17]. This is in agreement with a fumigation study carried out by Anderson and Molhave (1983) where they found that the death rate of spores of *Bacillus globigii* increased with formaldehyde concentrations ranging from 42,000 to 330,000 parts per billion (50,000 to 400,000 µg/m3)[18]. The hydrated form of formaldehyde, methelene glycol is the reactive component of formaldehyde[19]. Apart from the fact that reduction in the normal flora by the effect of formaldehyde can allow foreign pathogens to cause upper respiratory tract infections, the general effects of formaldehyde on the upper respiratory tract includes irritation of the eyes and nose, respiratory impairment, difficulty in breathing and so on[20]. Chronic exposure may cause burning and tightness of chest and some vegetative and neuro-behavioural changes including headache, nausea, and irritability[21].

In this study, the effects of formaldehyde on the normal flora of respiratory tract of rabbits and humans sampled were studied. Statistical analysis was done using student t-test to know the significant differences in these normal flora when exposed to formaldehyde. Some of the results showed statistical significance while others were not statistically significant. When the effect of formalin on throat flora of humans was compared between the test subjects and controls, it was statistically significant (p value < 0.05). Also, with nasal flora when compared, there was statistical significance. In addition, when the effect of formalin on the throat flora of rabbits was compared between the controls and those exposed to 10% formalin, it was statistically significant (p value < 0.05). But when compared between those exposed to 100% formalin and controls, there was no significant difference (p value > 0.05). This could be that formaldehyde is more effective as a disinfectant at lower concentration than at higher concentration. In animal study, *Staphylococcus species* dominated the nasal cavity. This is in accordance with a study conducted by Ajuwape et al., on the normal upper respiratory tract of puppies[22]. Also, a study conducted by Ajuwape and Aregbesola, revealed that the nasal bacterial flora of rabbits to contain both coagulase positive and coagulase negative *staphylococci*[23]. In accordance with Ajuwape and Aregbesola's study[23] where *Klebsiella species* was isolated, the same organism was also isolated in this study, but in contrast, *Micrococcus luteus* and *Pseudomonas aeruginosa* were not isolated. *Klebsiella species* dominated the throat of the rabbits. *Streptococcus species* were isolated in the throat of human subjects and no *Streptococcus species* in the nasal cavity of these human subjects. In contrast, no *Streptococcus species* was isolated in both the throat and nasal cavity of the rabbits sampled

which could be due to host specificity as a result of the presence of receptors on the cell surfaces that play important role in the adhesion of microbes to host cells. *Candida albican* was isolated from the throat of the rabbits and humans and non from the nasal cavity. Again the plausible explanation for this is the differences in host receptors in different tissues of the body.

Apart from the fact that formaldehyde causes reduction in the normal microbial flora, it could also be a potential agent to cause carcinoma in the nasal cavity of human beings. The most pronounced effect of formaldehyde is observed in the nasal mucosa, most of its carcinogenic effect is assumed to occur in the nasal cavity, although some indicate its effect in the oral cavity and pharynx[24]. These not definitely proven conditions are supported by the positive findings obtained from those in which rats were used[25].

In conclusion, it has been observed that formaldehyde can kill normal flora thereby resulting in reduction in number with the possibility of giving room for foreign pathogens to cause infection. It is therefore recommended that people that are at high risk of exposure to formaldehyde should try as much as possible to reduce their level of exposure. The histopathology laboratory, mortuary laboratory, embalming laboratory, histopathology cutting up room, and biology laboratory where tissues are fixed before sections are cut should be well ventilated and fume cupboard to extract this compound should be installed and monitored to work in order to reduce exposure to formaldehyde which has been shown clearly in this study to reduce the normal flora.

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