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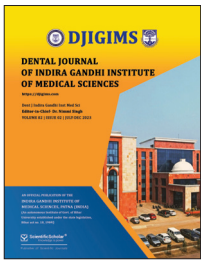

Original Article

Comparative Analysis of Cytomorphometric Parameters Among the Coconut Oil, Peanut Oil and Normal Saline in an Air-Dried Smears - A Switch to Routine Wet fixation

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ABSTRACT

Objectives: Rehydration of air-dried exfoliative cytology smears is considered as useful technique to increase the diagnostic accuracy of the air-dried smears and so the study had a point to determine and compare the cytomorphometric parameters of air-dried smears by rehydration using coconut oil, peanut oil and normal saline.

Material and Methods: A total of 135 smears were obtained from healthy volunteers and were air-dried. The smears were classified into three groups based on the rehydrating agent: coconut, peanut, and normal saline. The air-dried smears were rehydrated for a period of 5 minutes. And subsequently stained with Papanicolaou stain. The smears were studied under a microscope to evaluate cytoplasm and nuclear details and the quality of the background staining. Scores were assigned to each group and statistically analyzed using the chi-square test.

Results: All the studied parameters, such as cytoplasm, nucleus, and background, demonstrated high frequencies of superior scores in the peanut oil group, followed by the coconut oil group and the normal saline group. ($p < 0.05$)

Conclusion: Peanut oil is a rehydrating agent superior to coconut oil and can rehydrate the air-dried smears.

Keywords: Exfoliative cytology, Microscopy, Oral pathology, Peanut oil, Rehydration

INTRODUCTION

Exfoliative cytology is a diagnostic technique that is based on the microscopic examination of the epithelial cells that were exfoliated. It is considered a quick, non-invasive screening modality that enables oral pathologists to detect oral potentially malignant disorders and malignancies.[1] Cytological studies are routinely carried out using Papanicolaou (Pap) stain that is a valuable tool for studying the cytomorphometric changes associated with various inflammatory and malignant conditions.[2] Even though the technique of exfoliative cytology is simple and technically easy, it requires the cytological smears to be fixed right away in 95% ethanol and sent to a pathological laboratory for the smears to be stained and studied.[3] Delay in the fixation procedure can cause air drying artifacts and inadequate fixation, resulting in subpar staining and complicating the

diagnostic scenario. Additionally, poor fixation procedure also results in materialistic loss. The diagnostic inefficiency of the inappropriately fixed smears may lead to the repetition of smears to be taken from the patients, which increases the strain for both the clinicians and pathologists.[4]

To overcome these problems, rehydration is recommended as a possible solution.[3] Normal saline is considered a useful option for rehydration of smears owing to its wide availability in hospitals, clinics, and camps where several smears may need to be collected simultaneously for mass screening. The ideal period for air drying without sacrificing the success of subsequent rehydration is 30 minutes, while smears that have been air dried for up to a day can still be rejuvenated with rehydration, albeit with reduced success.[5] Natural agents such as coconut oil, peanut oil, bleached palm oil, etc., have been known to be useful in a histopathological laboratory.[6,7] According to evidence, coconut oil has been successfully used as a rehydrating agent in exfoliative cytology procedures.[3] Though various rehydrating agents are available, research analyzing their efficacy needs to be more extensive in the literature. The use of peanut oil in cytopathology has yet to be fully explored. Hence, our present study compared the cytomorphometric parameters of air-dried smears by rehydration using coconut oil, peanut oil and normal saline.

MATERIAL AND METHODS

A total of 135 smears were obtained from healthy volunteers randomly using a wooden spatula, in which three smears were prepared from each participant. The collected smears were air-dried and kept at room temperature for 48 hours. The smears were classified into three groups based on the rehydrating agent use, such as coconut oil (Group A), normal saline (Group B), and peanut oil (Group C), respectively. The rehydration procedure was done for a period of 5 minutes. Once the rehydration was done, the smears were subjected to staining with Papanicolaou stain and subsequently studied under the microscope at different magnifications by two experienced oral pathologists for the evaluation of nuclear and cytoplasmic details and the quality of the background staining.

The cytological smears were studied and scored above criteria were assessed based on the scoring criteria.[8] The cytological parameters were studied, and scores were assigned for the ease of studying the findings. Table 1 shows that the data obtained from the study was analyzed using Statistical Package for the Social Sciences (SPSS) Version 26. The chi-square test was used to assess the prevalence of cytological features among the three rehydrating agents used. A p-value < 0.05 was considered significant.

Table 1: Comparison of the scores of cytoplasm, nucleus, and background.

Variable		Based on the scoring criteria			P-value
		Score 0 n (%)	Score 1 n (%)	Score 2 n (%)	
Cytoplasm score	Coconut oil	0 (0)	22 (48.9)	23 (51.1)	0.001 Sig
	Peanut oil	0 (0)	13 (28.9)	32 (71.1)	
	Normal saline	18 (40)	22 (48.9)	5 (11.1)	
Nucleus score	Coconut oil	0 (0)	23 (51.1)	22 (48.9)	0.001 Sig
	Peanut oil	0 (0)	13 (28.9)	32 (71.1)	
	Normal saline	22 (48.9)	18 (40)	5 (11.1)	
Back-ground score	Coconut oil	5 (11.1)	16 (35.6)	24 (53.3)	0.001 Sig
	Peanut oil	0 (0)	18 (40)	27 (60)	
	Normal saline	18 (40)	22 (48.9)	5 (11.1)	

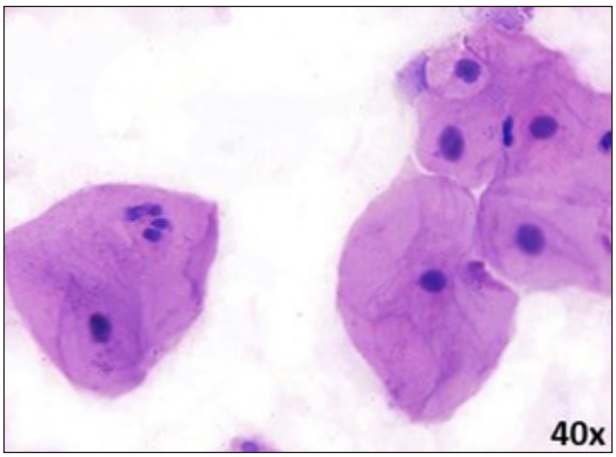

Figure 1: Papanicolaou (Pap) stained smear rehydrated with peanut oil, under 40× magnification, demonstrating a clear background with excellent nuclear and cytoplasmic staining.

RESULTS

Two independent oral pathologists studied all the smears. Cohen kappa test (κ) demonstrated a value of 1, indicating perfect agreement between the examiners who examined the smears. On examination of all 135 smears, All the studied parameters, such as cytoplasm, nucleus, and background, demonstrated high frequencies of superior scores in the peanut oil group as shown in Figure 1, followed by the coconut oil group [Figure 2]. The lowest scores were observed in increased frequencies in the normal saline group as shown in Figure 3, with p-values

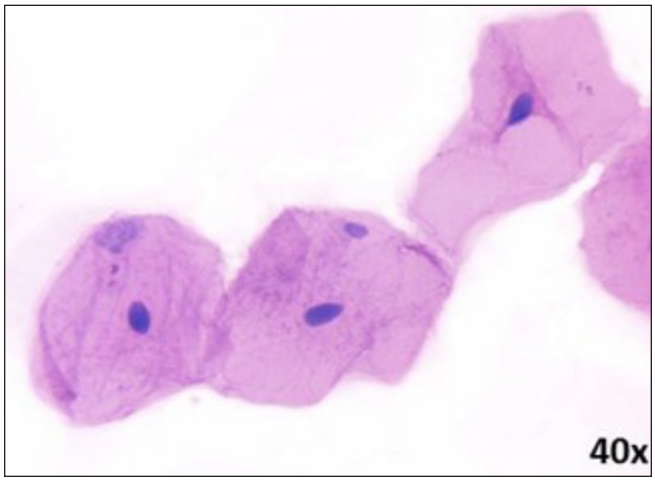

Figure 2: Papanicolaou (Pap) stained smear rehydrated with coconut oil under 40× magnification demonstrating good nuclear and cytoplasmic staining.

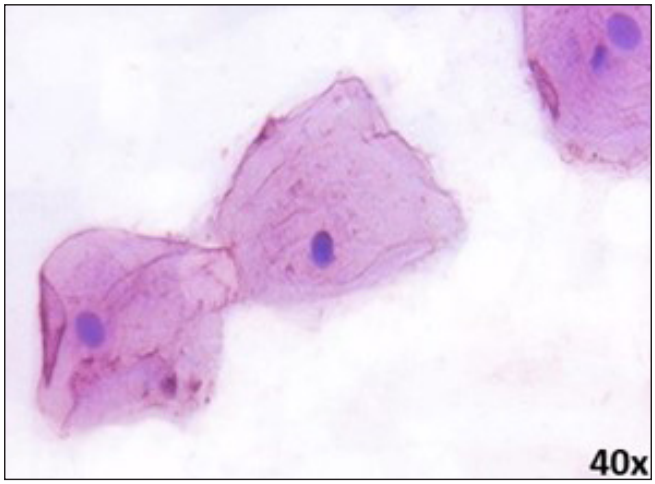

Figure 3: Papanicolaou (Pap) stained smear rehydrated with Normal saline under 40× magnification demonstrating fair nuclear and cytoplasmic staining.

of 0.012, 0.005 and 0.017, respectively. The difference was statistically significant ($p < 0.05$) [Table 1] [Figures 4-6]

DISCUSSION

Oral exfoliative cytology involves the procedures of examining and interpreting the characteristics of cells that have been exfoliated from the oral mucosa. It is a simple, non-invasive method that patients tolerate well and enables the establishment of an initial diagnosis for various illnesses.[8] Papanicolaou staining is employed as a standard cytological analysis method that helps study the basic inflammatory, dysplastic or malignant changes in the cells.[9] When smears are produced straight from the spatula, air drying of cytologic samples appears to be a factor that cannot be eliminated. In a certain way, mild drying is a prerequisite for the cells' adhesion to the slide's surface.[10]

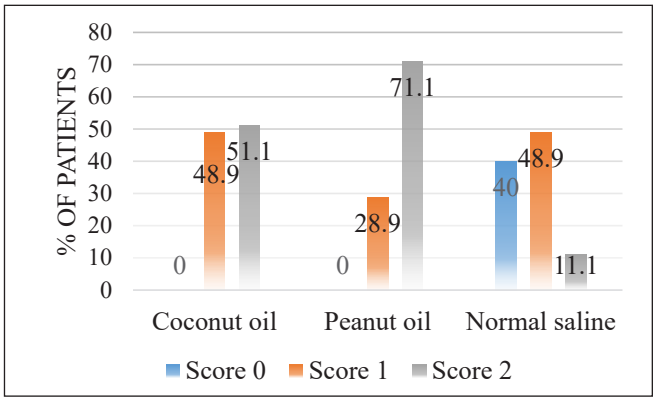

Figure 4: Graphical representation of cytoplasm scores of all three groups. Group C (peanut oil) shows good cytoplasmic staining properties compared to Group A (coconut oil) and Group B (normal saline).

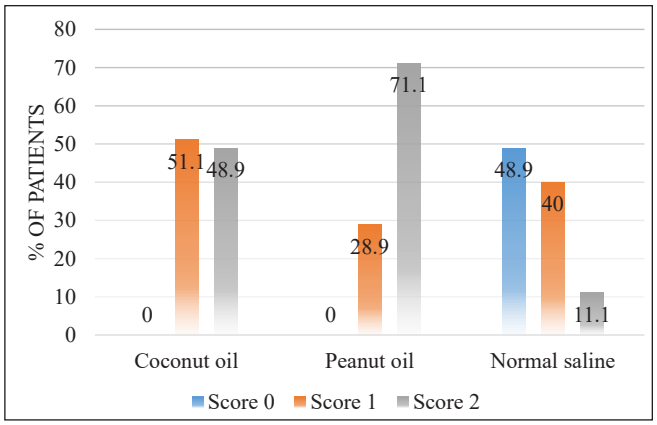

Figure 5: Graphical representation of nucleus scores of all three groups. Group C (peanut oil) shows good nuclear staining properties compared to Group A (coconut oil) and Group B (normal saline).

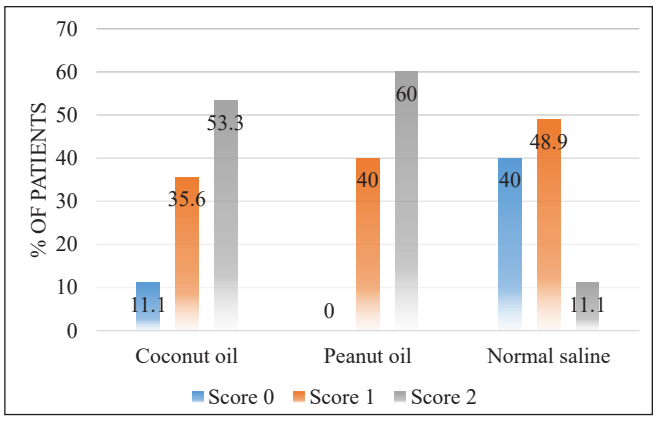

Figure 6: Graphical representation of background scores of all three groups. Group C (peanut oil) shows a clearer background compared to Group A (coconut oil) and Group B (normal saline).

Rehydration techniques are necessary to retrieve the diagnostic material from the smears that demonstrate drying artifacts. In pap smears, air-dried smears (ADS) have been the subject of contemporary research, especially regarding various rehydration procedures.[11] Sachin *et al*. utilized normal saline on ADS as an alternative to traditional wet fixed smears (WFS). They discovered nuclear and cellular features were better in ADS with rehydration than in conventional WFS.[12] Lencioni *et al*. performed the first rehydration of air-dried smears in 1950; they did it using tap water and an acetic acid-alcohol solution. For the same purpose in 1966, Bonime utilized 50% aqueous glycerine. Nieburgs utilized a coating of hydroxypropyl methylcellulose ether for the rehydration of smears.[13–15]

In our present study, we found the highest percentage of positive scores of cytoplasm, nucleus, and background in the peanut oil group. The scores of coconut oil were superior to normal saline but not to peanut oil. No studies have evaluated using peanuts as a rehydrating agent for air-dried oral smears. However, our study findings are similar to the findings of Antony *et al*. who reported coconut oil as an effective rehydrating agent for cytological examination of air-dried smears.[3]

Peanut oil is an edible vegetable oil with high rancidity strength, non-toxic, safe in heat, and oxidizes slowly. Eight different fatty acids comprise most of the triglycerides in peanut oil. Oleic and linoleic acids make up about 80% of these fatty acids. These two fatty acids typically change oppositely. Linoleic acid is the major polyunsaturated fatty acid that makes up peanut oil's chemical composition.[16,17] Peanut has been studied as an alternative clearing agent to xylene in histopathology.[16,18]

Coconut oil contains 92% saturated fats and is mostly made up of Medium Chained Fatty Acids (MCFAs). Lauric Acid (LA) accounts for 50% of the composition of coconut oil. Other minor constituents in coconut oil include caprylic acid, myristic acid, palmitic acid, capric acid, oleic acid, stearic acid, and linoleic acid.[5] Normal saline is an age-old option considered a rehydrating agent for treating air-dried smears because of its wider availability in hospital settings and camps. Smears undergo air drying while being transported without tools and assistance like jars or fixatives. The ideal amount of time for air drying without sacrificing the success of subsequent rehydration is 30 minutes, while smears that have been air dried for up to a day can still be saved with rehydration, albeit with reduced success.[6]

Rehydration of air-dried smears is a simple and reliable fixation approach comparable to the conventional wet fixation procedure used for cervical smears. It can be routinely used in diagnostic cytopathology. Various ways for rehydrating ADS have been described in several investigations since the 1950s.[19] A study by Rupinder *et al*. reported excellent cytomorphological preservation in WFS compared to ADS.[20] Hence, Wet Fixation procedures are superior, yielding excellent diagnostic details of cytological smears.

Literature evidences has reported peanut oil and coconut oil as suitable alternatives to xylene owing to their non-hazardous nature, less expensive, and results in less tissue shrinking. Without affecting the quality of the histological details, it can be employed as a clearing agent in the histopathology laboratory.[21]

This present study is an attempt made by us to investigate the rehydration efficacy of peanut oil. However, our study has an inherent limitation due to a small sample size. Further large-scale studies should be undertaken to investigate and establish the properties of peanut oil as a rehydrating agent in cytological examination.

CONCLUSION

Peanut oil is a natural agent and edible oil that has the potential to be used as a rehydrating agent, and it is found to be superior to coconut oil in terms of rehydration of air-dried smears. Hence, peanut oil can be an excellent alternative to normal saline, commonly used in air-dried smears' rehydration procedures.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation

The authors confirm that there was no use of Artificial Intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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