

Comparison of Honey with Alcohol as a Fixative in Fine Needle Aspiration Cytology

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Abstract: Fine Needle Aspiration Cytology (FNAC) is a useful diagnostic aid in benign and malignant lesions. The diagnostic accuracy of FNAC depends on the sampling technique, fixative used, quality of staining and of course the meticulousness of interpretation by the pathologist. The widely used fixative in this procedure is 95 % Alcohol which although readily available, has a number of limitations for use. It is expensive and hence subject to pilferage. Also, it is a volatile liquid with an irritant smell and is said to be carcinogenic. Recent attempts have been made to prove that honey is also a comparable fixative for cytological specimens. It is a natural organic product, odourless and not known to cause any disease. Secondly it is relatively cheaper and not prone to be mishandled, making it an efficient contender for use as a fixative. In this study, we analyzed and compared the efficacy of cytological smears fixed in 95% alcohol and 20% honey solution. The results of our study showed no statistical significant difference in the fixative properties of honey and alcohol. Our results, therefore, confirmed that honey can safely be used as an alternate to alcohol in cytology specimens.

Introduction

The word "diagnosis" is of greek origin. "Dia" meaning **through** and "gnosis" meaning **knowledge**. So, diagnosis essentially means determining the possible cause and nature of a patient's disease through clinical and laboratory knowledge.¹ In surgical pathology, there are two basic diagnostic techniques i.e. Histopathology and Cytology. Cytology is further divided into Aspiration cytology and Exfoliative cytology.² The accuracy of diagnosis in both these techniques depends very much on pre-analytical factors such as the sampling technique, quality of fixation and staining. All these factors pave way for the pathologist to correctly interpret the microscopic findings.

The history of fixation is quiet ancient, going back to the time of Egyptians and Hippocrates. Hippocrates studied the effects of mercury and alcohol as fixatives in 400 B.C.³ Then came the microscopic era in early 1620s, and a whole new world, since then invisible to the naked eye was revealed. The early microscopists however were not actually interested in morphological details of tissues and cells but were rather enthralled by anything new that they saw under the lens.

With advancements in the field of medicine and diagnostics, surgical pathology was accepted as an important diagnostic tool. Therefore, something was needed that could preserve the tissues and cells in as "life like" state as possible or in other words "fix" them. A number of substances are currently in use as fixatives. These include formalin, alcohol, bouin, carnoy, muller fixative and the list goes on. But, as much as it has been searched for, there is no ideal fixative that fulfills the all the requirements of cell or tissue preservation, till date.²

The fixative widely in use as a cytological fixative is 95% alcohol. It is an excellent, proven fixative due to many properties such as rapid action, efficiency of fixation and wide applicability. However, it is an expensive, volatile and flammable liquid with an irritant smell. It has also been shown to be carcinogenic in some animal models. Alcohol acts as fixative by denaturing proteins present in tissue and by dehydrating them. The dehydration may cause shrinkage of the tissue, which is yet another limitation to its use. Furthermore, the lipids present within the tissue are washed out by alcohol.⁴

Honey has been in use as a fixative since ancient times. It is known that Egyptians used it during the process of embalming of dead bodies. According to a legend, body of Alexander the great was preserved in honey for two years before being buried.⁵ Codex alimentarius defines honey as "**A natural sweet substance, produced by honeybees from the nectar of plants,**

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which the bees collect and transform by combining with specific substances of their own. It is then deposited, dehydrated, stored and left in honeycombs to ripen and mature.”⁶Honey has antioxidant, antimicrobial, antiautolytic and tissue hardening properties. The antibacterial effect of honey is due to osmotic effect, acidity and generation of hydrogen peroxide.⁷ Compared to alcohol, it is non-hazardous, natural organic product, is odourless and does not require additional equipment. However, limitations to its use include viscosity and growth of molds when left over a period of time.

Previously, few studies have been done to evaluate the potential replacement of formalin with honey and other natural sweeteners. Though most of these studies have been done on histopathological specimens, recent attempts have been made to prove that honey is a comparable fixative for cytological specimens also. In this study, we analyzed and compared the efficacy of cytological smears fixed in 95% alcohol and 20% honey solution..

Vitamin B-12 deficiency is a worldwide problem, however, particularly in the newborn period, due to the combined effects of poor maternal diet and congenital deficiencies of transcobalamin.^{7,8} These underprivileged infants who are exclusively/ predominantly breastfed tend to develop B12 deficiency as the breast milk content of B12 in these mothers is far below normal. Cobalamin content of breast milk is lower in vegetarian mothers and is positively correlated with their serum cobalamine levels.^{5,6} Prognosis depends on the underlying cause of the megaloblastic anemia and the degree of compliance with therapy. Folic acid deficiency is relatively easy to treat.

Materials and Methods

30 cytological smears were randomly selected from patients coming for FNAC (from November 2015-January 2016) at Department of Pathology, Pakistan Institute of Medical Sciences. Patients of all age groups and co-morbids were included. Informed consent was taken from patients. The honey solution was prepared by mixing 40 ml of honey in 160 ml of distilled water (20 % v/v solution). Two attempts at FNAC were made, using the standard protocol for this procedure. Out of the two slides prepared by one attempt, one was fixed in alcohol and the other in honey solution. All slides were fixed for a minimum of 10 minutes. Slides were then allowed to dry and stained with Hematoxylin and Eosin stain (H & E). Slides were then

examined blindly by two post graduate trainees and a consultant histopathologist. Scores were allotted according to following criteria. (See table)

TABLE FOR SCORING CRITERIA⁴

Features	Scores and criteria	
Nuclear staining	Acceptable (1) : round, smooth and clear nuclear membrane	Unacceptable (0) : granular, disintegrated and out of focus
Cytoplasmic staining	Acceptable (1) : intact cytoplasmic membrane and transparent cytoplasm	Unacceptable (0) : disintegrated membrane, granular cytoplasm and out of focus.
Cell morphology	Preserved (1) : Absence of folds, no overlap, maintained N/C ratio	Unpreserved(0): Overlapping cells, folded and disintegrated cells
Clarity of staining	Present (1): Crispiness in staining and transparency	Absent(0): Obliterates the nucleus and cytoplasm
Uniformity of staining	Present (1): Uniformly stained throughout the individual cells	Absent (0): Stained in different shades of colour in individual cells

Adequacy criteria:

For thyroid and breast lesions, the standard protocol was followed i.e. **at least six clusters of cells** in each slide, each cluster comprising of 8-10 cells.

For all other lesions, the slide was considered **adequate** if it contained more than 40 cells and **inadequate** if less than 40 cells were present on slide.

Data Analysis

The scores of both smears fixed in alcohol and honey were compared for all individual parameters. The results were analysed by using SPSS version 21 and paired sample T-test was applied for significance. A value of <0.05 was considered significant.

Results

Out of the 30 studied cases, only clarity of staining was absent in 3 (10%) cases fixed in alcohol. Rest of the parameters were fulfilled in all (100%) of the alcohol fixed cases. For 30 cases fixed with honey, 2 (8%) showed unacceptable cytoplasmic staining and unpreserved cell morphology. Clarity of staining was absent in 6 (25%) cases. After applying paired sample T-test p-values were obtained for all parameters: nuclear staining = 0.16; cytoplasmic staining = 0.16; cell morphology = 0.16; clarity of staining = 0.006; uniformity of staining = 0.66. Overall, the average percentage of acceptable parameters fixed with alcohol

was 98% compared with 92% of cases fixed with honey. The results showed no statistical difference in the fixative properties of alcohol and honey.

Parameter	Alcohol Results N= 30	Honey Results N= 30	P Value
Nuclear Staining	30 (100%)	30 (100%)	0.66
Cytoplasmic Staining	30 (100%)	28 (93%)	0.16
Cell Morphology	30 (100%)	28 (93%)	0.16
Clarity Of Staining	27 (90%)	23 (77%)	0.006
Uniformity Of Staining	30 (100%)	30 (100%)	0.66

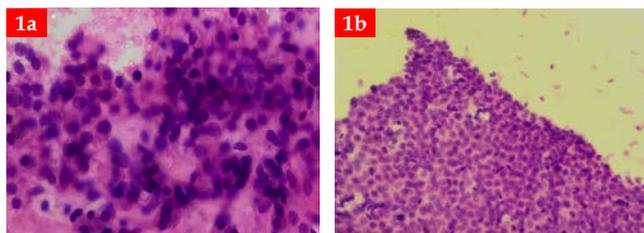


Figure 1a and 1b show thyroid follicular cells. Cytoplasm in 1a (Honey fixed) is granular and relatively less clear than 1b (alcohol fixed)

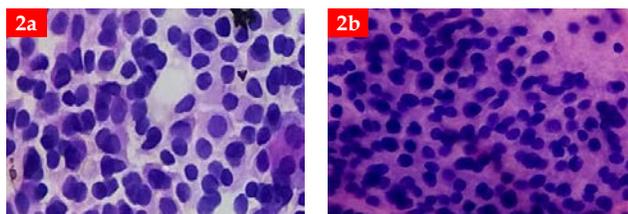


Figure 2a (honey fixed) and 2b (alcohol fixed) show ductal epithelial cells showing equally good morphology

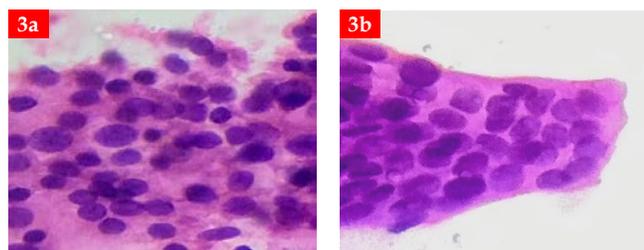


Figure 3a (honey fixed) shows more eosinophilic cytoplasm as compared to 3b (alcohol fixed)

Discussion

The basis of good microscopy is proper treatment of tissue/cells after their removal from the body. For this purpose they should be fixed as soon as possible. Fixation works by stabilizing the tissue elements and preserving their morphological details. A number of fixatives are currently in use; all of them working on

the principle of cross-linkage of cellular proteins.⁹ For cytology, Alcohol is the most commonly used fixative. Owing to its irritant smell and potential harmful biological effects, studies have been done recently implicating the role of honey as a fixative.

A study done on cytological smears showed Singh et al. showed no difference in the parameters studied in honey and alcohol, concluding that honey is as efficient as alcohol as a fixative. Most of the other studies have been done on the histopathological specimens. Lalwani et al showed no significant difference in fixation properties of honey and alcohol in histopathology specimens of oral tissues. Our results are in conformation with previous studies proving honey fixes cytology specimens almost as good as alcohol does. Further studies are still needed, especially with larger sample size to establish this finding.

Out of the five parameters that were compared for fixative qualities, only clarity of staining showed a significant difference on in alcohol and honey. The clarity was significantly lower in honey stained smears, The reason for this is not known, but may be attributed to the viscous nature of the fluid.

Pertaining to cytoplasmic staining as assessed by transparency of cytoplasm and integrity of membrane, two of the honey fixed slides did not fulfill the adequacy criteria, since the cytoplasm of honey fixed slides was more granular and not homogenous as compared to alcohol fixed slides. But the difference was not statistically significant ($p=0.16$) (fig 1a& 1b). The granularity may have something to do with the constituents of honey as it contains many minerals and trace elements which could interact with cytoplasmic constituents. Cellular morphology was also unpreserved in two of honey fixed slides, but again the difference with alcohol was statistically insignificant ($p=0.16$) (fig. 2a& 2b). Additionally the background of honey fixed slides was clearer and the cells fixed in honey showed more eosinophilia (fig. 3a& 3b). The latter problem was solved by decreasing staining time with eosin.

A few drawbacks of the study include growth of fungus in the honey solution when left over a period of time. Also the pathologists trained to look at cytology slides fixed in alcohol may find it difficult to interpret specimens fixed in honey.

Conclusion

The comparable performance of honey identified in our study advocates its safe use as a fixative in

cytological specimens. However, honey as a fixative has its limitations for use. We know there is no ideal fixative and every fixative has its pros and cons so the use of a particular fixative depends on multiple factors. Further studies may be needed especially on cytological specimens to make a definitive conclusion. To sum it all "honey does not solve all problems but then, neither does alcohol."

References

1. Rai R, Bhardwaj A, Verma S. Tissue Fixatives: A Review. International journal of pharmaceuticals & drug analysis. 2016;4(4): 183-7
2. Grizzle WE .The Use of Fixatives in Diagnostic Pathology. The Journal of Histotechnology. 2001;24(3):151-2
3. Sabarinath B, Sivapathasundharam B, Sathyakumar M. Fixative properties of honey in comparison with formalin. Journal of Histotechnology. 2013;37(1):21-5
4. Singh A, Hunasgi S, Koneru A, Vanishree M, Ramalu S, Manvikar V. Comparison of honey with ethanol as an oral cytological fixative: A pilot study. Journal of Cytology. 2015;32(2):115-7
5. Stiff RM. The Curious Lives of Human Cadavers. Paw Prints. 2010:52-3.
6. Olaitan PB, Adeleke OE, Ola IO. Honey: A reservoir for microorganisms and an inhibitory agent for microbes. African Health Sciences. 2007;7:159-65.
7. Patil S, Rao RS, Ganavi S, Majumdar B. Natural sweeteners as fixatives in histopathology: A longitudinal study. Journal of Natural Science, Biology and Medicine. 2015;6(1):67-70
8. Özkan N , ES Alva , Akala g̃ ao g̃ lu FC , üzüner BT. Honey as a substitute for formalin?. Biotechnic & Histochemistry. 2012;87(2):148-153
9. Culling CFA, Allison RT, Barr WT. Fixation. Butterworth and co. Ltd. Cellular Pathology Technique. 1985. p.27

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