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Letter to the Editor

Comparison of formalin and FineFIX in preserving DNA material in small biopsies

To the Editor:

Formalin is a routine fixative in surgical pathology wards. Although morphological assessment is very desirable in formalin-fixed materials, DNA and RNA are not preserved well and molecular studies cannot be performed with reliable results. Biopsies and surgical specimens, as well as post-mortem tissue samples, represent an important resource, especially for studies of molecular epidemiology, rare disease, neuropathology, and case studies with very long follow-up periods.[1] A major advantage of using paraffin-embedded material is that it is easier to collect tissues from already existing archives in comparison to frozen fresh tissues that need a specific sample collection, with dedicated spaces and specific equipment.[1] Because formalin fixation results in poor preservation of DNA and RNA, there are efforts to introduce new molecular friendly fixatives by some investigators.[2,3]

Recently a new fixative named FineFIX, which is ethanol based, has been introduced (Milestone, Sorisole, Italy) but few published articles exist about its advantages in improving molecular analysis.[1]

Nowadays many small samples are taken during endoscopy or needle biopsies and sent to pathology laboratories, and these may be the only available specimen for future molecular studies. We found no published data on DNA studies in small samples, so we decided to examine this fixative at a molecular level and compare it with formalin in small biopsies.

Ninety small gastric biopsies, similar in size, taken during endoscopy, were put in formalin and in FineFIX in equal numbers, in our laboratory (Pathology Department, Children Medical Center affiliated with Tehran University of Medical Sciences). Due to the small size of the biopsies we could not divide each sample into two halves. Duration of fixation was equal for the two groups.

Standard processing was performed, and HE- and Giemsa-stained slides were prepared for each case, and those of unsuitable quality and quantity were excluded from the study.

The morphology of two groups was compared among the two methods. Seventy biopsies were selected for the study (20 samples were excluded because they were superficial or very small).

DNA was extracted from 5 μm sections of both FineFIX and formalin fixed-paraffin wax-embedded tissues, until no tissue remained in the blocks (Genomic DNA extraction kit, Cat. No: k3032, Lot No: 0602; Bioneer Inc., Alameda, CA, USA). Each sample was amplified using specific primers for human β_2-microglobulin. Polymerase chain reaction conditions (PCR) and amplicon lengths are listed in Table 1.

Polymerase chain reaction (PCR) was performed in a 50 μL final volume using standard conditions (Dynamic BioSciences (PouyaZistech), Tehran, Iran; kit Cat. No. KP175). Every reaction included 50–100 ng of DNA, 2 μL of each primer, which were added to lyophilized mastermix composed of KCl, MgCl2, Tris HCl, dNTP and Taq DNA polymerase.

The following amplification program was used for every PCR reaction analysis: initial denaturation at 94°C for 3 min; 40 cycles composed of denaturation, at 94°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 1 min. Final extension was performed for one cycle for 3 min at 72°C. Statistical analysis was performed using χ^2 test for comparison of the two groups.

Morphological studies of the two groups indicated shrinkage of tissue in the FineFIX group, which is undesirable but does not interfere with final diagnosis (Fig. 1). Mast cell count in gastric biopsies, which is performed routinely in our lab for Giemsa-stained slides, could not be done due to degranulation of mast cells or staining quality in the FineFIX group, which has not been previously reported.

In the formalin-fixed group 20 of 35 specimens had a β_2-microglobulin band (248 bps), but 30 out of 35 had this band in the FineFIX group (Fig. 2). The difference between these two groups in preserving DNA for this gene was statistically significant ($P = 0.008$). The PCR reaction for 10 specimens with no amplification product was repeated but the results were the same.

Although formalin is a good preservative for morphological purposes, molecular study, especially in small biopsies, is not

Table 1 Polymerase chain reaction conditions

Gene	Primer sequence	Length of product (bp)	Temperature of annealing (°C)	Cycles
β_2-microglobulin	HMBB01 (Forward) GCG ACC CAA TGC AAA TTG GT PC04 (Reverse) GAA GAG CCA AGG ACA GGT AC GH20 (Reverse) CAA CTT CAT CCA CGT TCA CC	248	54	40

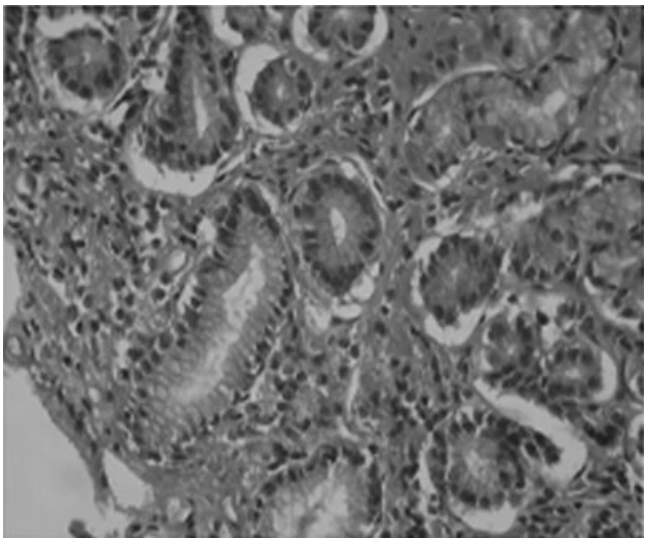

Figure 1 Tissue fixed in FineFIX (Milestone) with slight shrinkage artifact (hematoxyline eosin stain, X400).

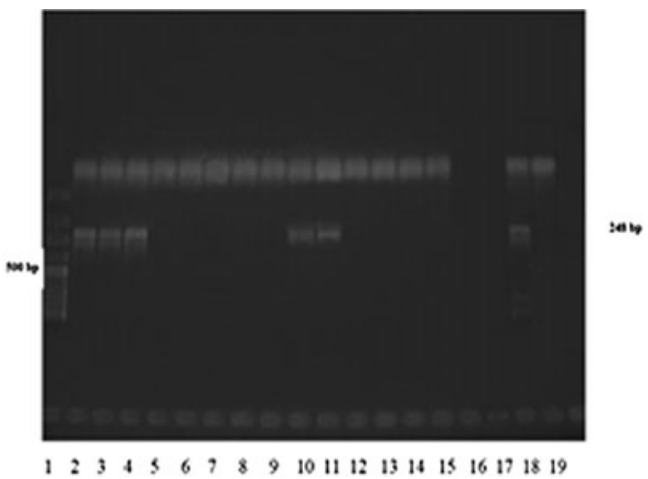

Figure 2 Polymerase chain reaction of DNA from formalin and FineFIX tissues from left to right: lane 1, ladder; lanes 2–7, FineFIX; lanes 8–15, formalin; lane 18, positive control; lane 19, negative control (ethidium bromide staining).

desirable. Other fixatives such as FineFIX may be substituted. According to Sambrook *et al.* the time-honored formalin fixation and routine tissue processing methods are of limited, if any, value in preservation of biomolecules.[4] Ideal tissue fixation and processing procedures should preserve histomorphological features, similar to that seen in formalin-fixed paraffin-embedded material, while protecting DNA, RNA, and protein in a manner comparable to fresh-frozen tissue. This will enable the pathologist to render a histological diagnosis and use the same tissue slide, or corresponding paraffin block, to isolate intact macromolecules, hence allowing retrospective molecular studies on archival histological material.[2] Lewis *et al.* have suggested that fragments longer than 200 bp could not be reproducibly amplified from the formalin-fixed material.[5]

Several investigators have introduced novel molecular friendly fixatives in recent years such as UMFIX.[2] The present study was performed with a new fixative named FineFIX introduced by Italian investigators,[1] which is widely distributed in Iran. There is only one article, however, about the benefits of this fixative published by the same investigators. Because we receive many small biopsies daily, we wanted to assess if FineFIX is superior over formalin in preserving DNA in small samples. The present results indicate that this fixative is superior to formalin in this regard and the difference was statistically significant. Although most investigators have claimed that morphology and other techniques such as immunohistochemistry, western blot and proteomics can be done with the same or even better results using FineFIX or other ethanol-based fixatives,[2,3,6] in the present study the morphology of the specimen was better in formalin-fixed tissues. This may be due to bias, given that we have been using formalin-fixed slides for many years.

There are also studies on RNA preservation in ethanol-based fixatives and paraffin-embedded tissues,[2,3,7] but RNA study was not aim of the present study.

In recent decades the trend has been to take a smaller amount of biopsy material by needle or other means, therefore there are more small biopsies than in the past, hence it is wise to use molecular friendly methods of fixation for these small samples. Study using larger series and different tissues supported by complementary tests are recommended for better comparison of these two fixatives.

In summary, we highly recommend use of fixatives such as FineFIX in institutions where molecular study is favored, especially in research centers.

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