Acid-free glyoxal as a fixative for fresh histological tissue samples

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MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS

Introduction

Formalin fixation and paraffin embedding (FFPE) remains the standard method for tissue processing worldwide. However, some of the tissue specimens must be dissected and examined fresh, and the use of formalin for transport and preservation is not optimal due to formalin exposure, prolonged fixation and limitation of DNA and RNA quality. Therefore, novel approaches in the preanalytical phases of tissue handling are needed.

The aim of this study was to analyze the suitability of a new acid-free glyoxal (GAF) as a fixative for fresh histological tissues because of its good chemical properties and ability of safe use.

Methods

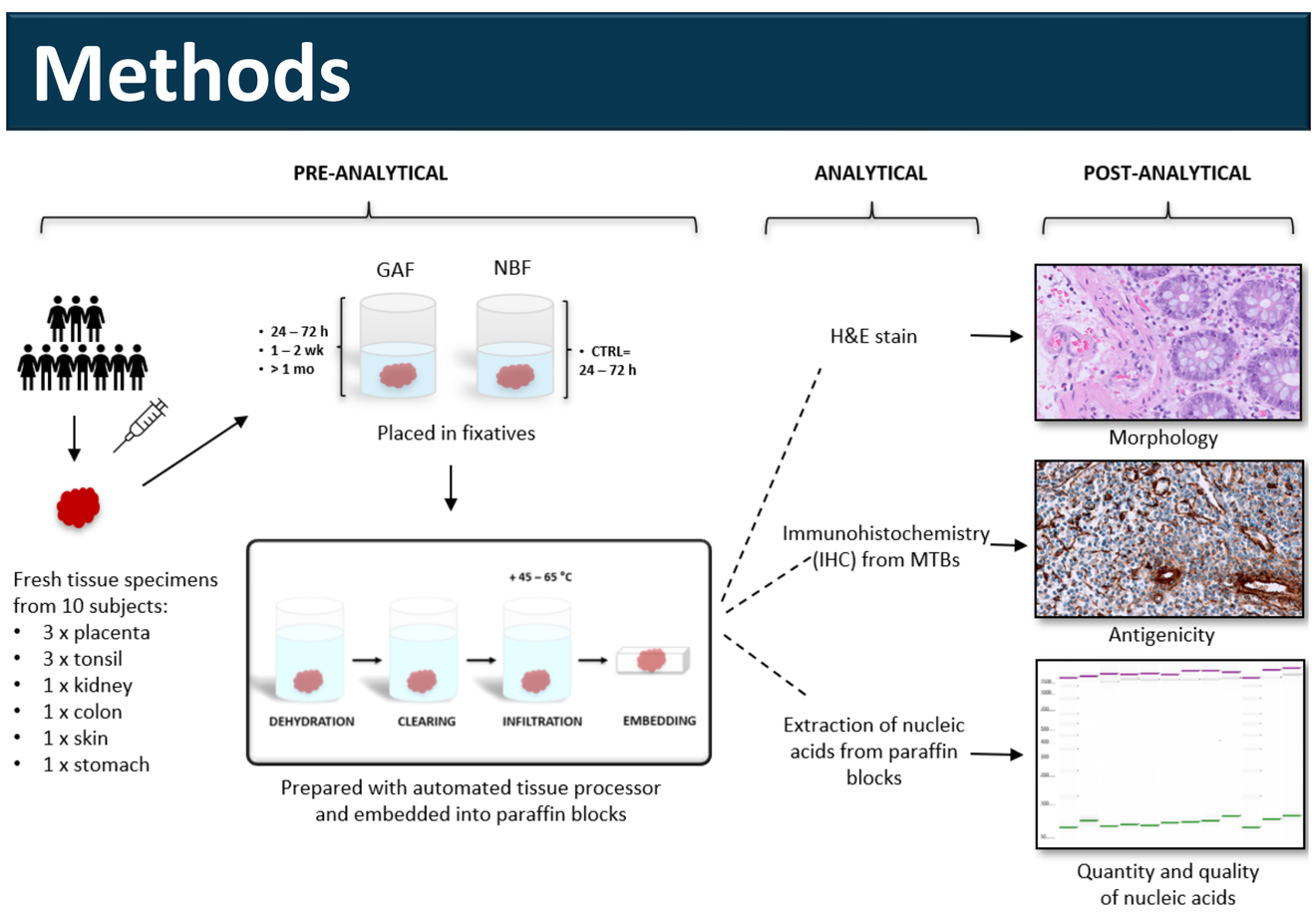

Figure 1. Workflow of the master thesis research. (GAF) Glyoxal Acid Free; (NBF) Neutral Buffered Formalin; (MTB) Multi Tissue Block.

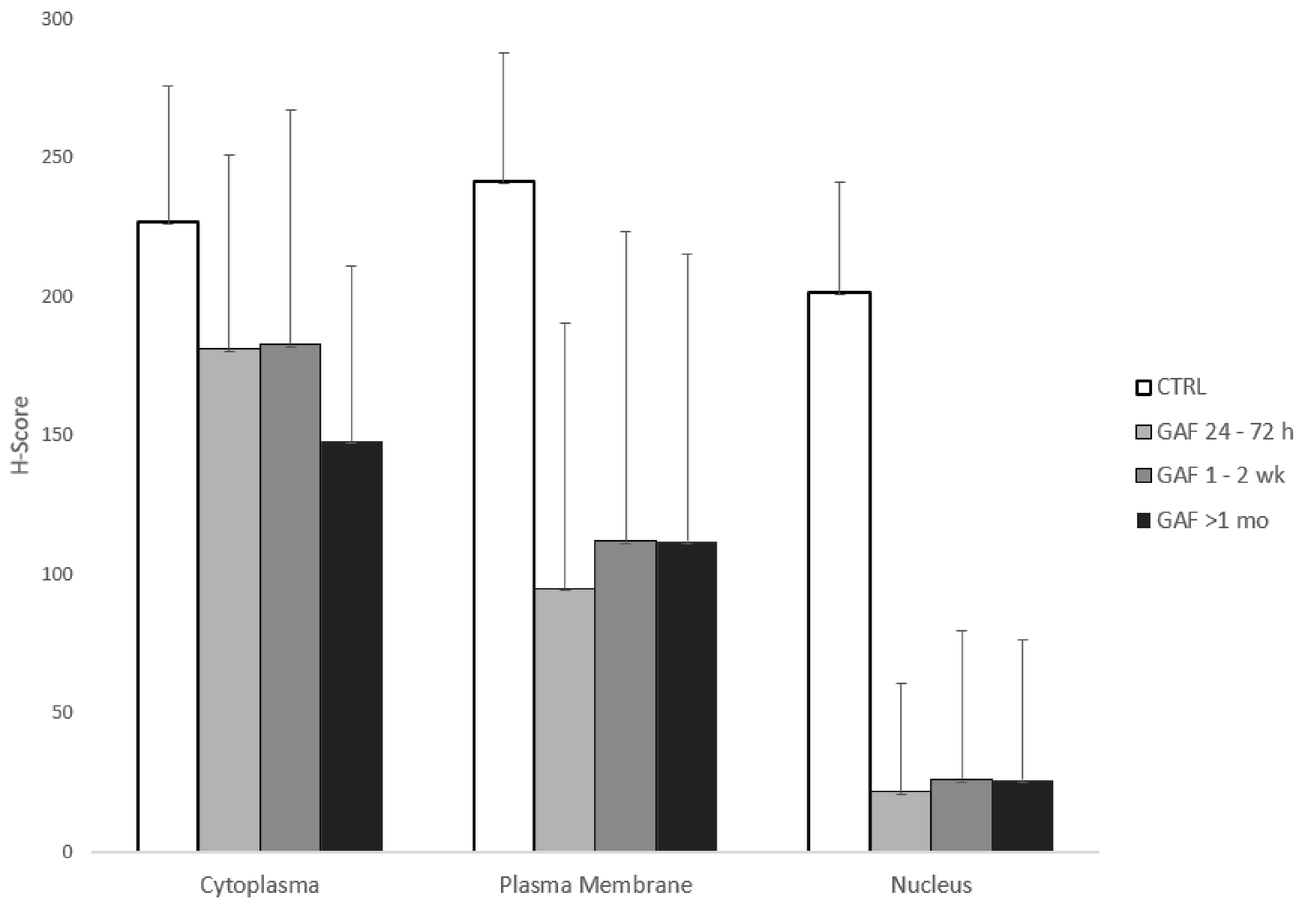

Figure 4. Preservation of antigens in GAF-fixed tissues. HistoScore was counted with following equation: H-score= (1 × (% of weak staining) + 2 × (% of moderate staining) + 3 × (% of strong staining). The score ranges from 0 – 300. (Wen et al. 2023).

Results

Figure 2 shows the morphological quality in GAF-fixed placenta (B), which was comparable to FFPE control tissue (A). The quality remained relatively stable and the colour less eosinophilic after a longer duration of fixation (C-D). Similar results were obtained from skin, stomach and kidney. Shrinkage of tissue occurred in tonsil and colon (Figure 3). Immunohistochemistry revealed good preservation of cytoplasmic antigens when fixed for less than 2 weeks (Figure 4). Plasma membrane and nuclear antigens were poorer preserved. The molecular analyses showed enrichment in DNA fragment size and less RNA degradation in GAF-fixed tissues compared to FFPE-processed samples.

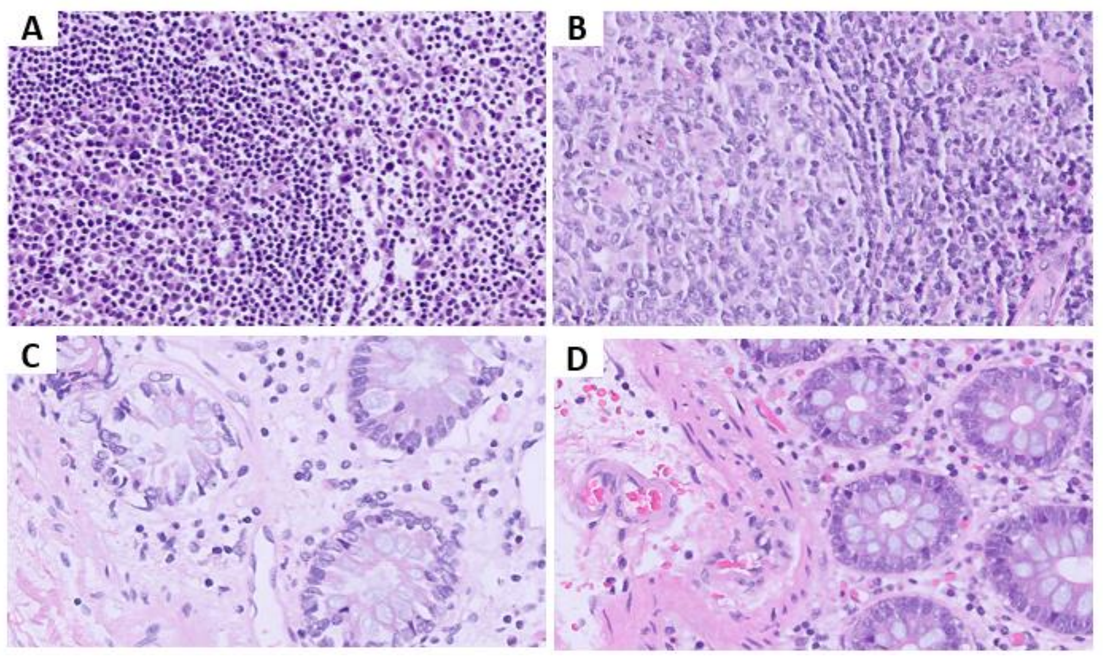

Figure 3. Morphology of GAF-fixed tonsil and colon. (A) GAF-fixed tonsil; (B) FFPE tonsil; (C) GAF-fixed colon; (D) FFPE colon. Duration of fixation is 24 – 72 h in each sample.

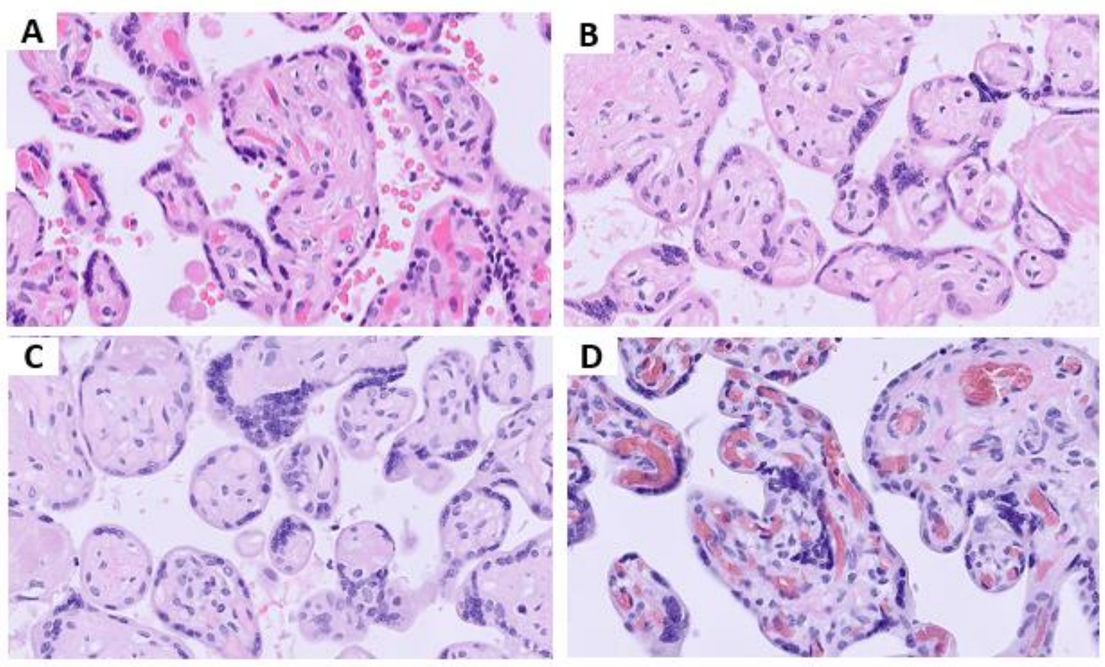

Figure 2. Morphology of GAF-fixed placenta for different durations. (A) FFPE placenta (control sample); (B) GAF-fix for 24 – 72 h; (C) GAF-fix for 1 – 2 weeks; (D) GAF-fix for > 1 month.

Discussion

The good histological quality and enhanced preservation of nucleic acids suggest the potential role of GAF in molecular pathology. However, the IHC results of GAF-fixed tissues were inferior compared to those with the gold standard formalin. Since all histological procedures in the pathology laboratory are optimized for formalin, these procedures should be adjusted for glyoxal-fixed tissues and further studied.