ORIGINAL RESEARCH

Comparative Evaluation of Efficacy of *Kumkum* as an Alternative to Periodic Acid in PAS Staining

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Abstract

Introduction: Stains are a crucial component of laboratory procedure. They help in highlighting the different tissues that plays an important role in diagnostic histopathology. Synthetic dyes are utilised for majority of the stains in histology which are considered to be harmful for the laboratory technician and pathologists. Periodic acid on the queue, is considered to be hazardous and is classified under OSHA Class-II category (Highly inflammable), which is on the stake of consideration. Among the natural surrogates studied, *Kumkum* has equivalent oxidizing property and is comparatively a safer option.

Aim: To compare the staining characteristics of *Kumkum*, which oxidizes the 1,2 glycol group as an alternative to Periodic acid in PAS staining.

Materials and Methods: Two study groups were determined as Group A & Group B. A total of 30 tissue blocks of suitable cases were obtained, each was made into two sections. Group A was stained with routine Periodic Acid Schiff Technique, while Group B was stained with *Kumkum* solution and Schiff reagent. The Cellular Architecture (CA) and quality of staining of both the groups were compared and a scoring system was given. The scores were analysed with Chi-square test using SPSS software.

Results: This study hypothesizes that *Kumkum* as a surrogate to Periodic acid will lead to a significant improvement in staining and is less hazardous.

Conclusion: This study is the first of its kind to have used *Kumkum* stain for the differentiation of glycogen and mucin components in histostaining of oral tissues. The naturally prepared *Kumkum* stain possesses dual staining property both in routine and differential staining, facilitates that naturally obtained *Kumkum* is a safer option and a better substitute for periodic acid.

Keywords: Utility of *Kumkum,* Natural alternative, Oxidation.

Introduction

Histopathological diagnosis remains the cornerstone of definitive disease identification, particularly in complex fields like oral pathology where distinguishing benign reactive lesions from malignancies or identifying specific entities (e.g., mucocele, fungal infections, and glycogen storage disorders) is paramount. Staining techniques are indispensable tools in this process, enhancing contrast and revealing specific cellular and extracellular components invisible in routine Hematoxylin and Eosin (H&E) preparations. Among these, the Periodic Acid-Schiff (PAS) reaction stands as a critical histochemical method. Its principle relies on the oxidation of 1,2-glycol groups present in carbohydrates by periodic acid, resulting in the end product di-aldehydes. These di-aldehydes then react with Schiff's reagent, producing a characteristic magenta or purple-red colour. This makes PAS invaluable for visualizing a wide array of structures, including glycogen, neutral mucin, glycoproteins, glycolipids, basement membrane, and fungi[1].

However, periodic acid – presents significant occupational health and safety challenges. Classified by the Occupational Safety and Health Administration (OSHA) under Class II (Highly Flammable), periodic acid poses substantial risks. Its highly flammable nature necessitates stringent storage and handling protocols away from ignition sources. It is corrosive to skin, eyes, and respiratory tract, requiring personal protective equipment (PPE) including gloves, goggles, and fume hoods. It has the potential for hazardous reactions with incompatible materials. Long-term exposure, even at low

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levels, raises concerns for laboratory technicians and pathologists who handle it daily. While specific chronic effects are less documented than acute ones, the inherent hazard classification warrants minimizing exposure. Safe disposal requires specific procedures to avoid environmental contamination[2].

The ongoing reliance on periodic acid, despite these risks, underscores the lack of readily available, equally effective, and safer substitutes. This has spurred research into natural alternatives with oxidizing capabilities. Turmeric, and specifically its traditional derivative Kumkum- a mixture of Curcumin and slaked lime, has shown promising results due to its inherent oxidizing properties. Curcumin, the principal curcuminoid in turmeric, possesses a di-ketone structure capable of undergoing redox reactions, potentially mimicking the glycol-oxidizing function of periodic acid. Kumkum offers several theoretical advantages: Derived from readily available botanical sources[4]. Generally regarded as safe for topical and limited internal use in traditional practices, suggesting a potentially lower hazard profile compared to strong inorganic acids like periodic acid. Potentially lower cost than synthetic periodic acid, especially in regions where turmeric is abundant. Preliminary observations suggest Kumkum-based solutions might offer staining nuances beyond simple oxidation[5].

This study directly addresses the critical need for safer PAS staining in oral pathology laboratories. We hypothesize that Kumkum solution can effectively substitute periodic acid in the PAS reaction for oral tissues, providing comparable or superior staining quality for glycogen and mucins, while offering a significantly improved safety profile. This investigation represents the first application and evaluation of Kumkum specifically for histochemical staining differentiation in oral tissues6.

Aim

To comprehensively compare the staining characteristics, efficacy, and quality of Kumkum solution against standard periodic acid in the PAS technique for the visualization and differentiation of glycogen and mucin components in routinely processed oral tissue sections.

Materials and Methods

Study Design: A prospective, controlled, laboratory-based study comparing two staining techniques on paired tissue sections.

Tissue Selection and Processing:

Archived formalin-fixed, paraffin-embedded (FFPE) tissue blocks were retrieved from the Department of Oral Pathology archives. Blocks were selected from cases known to contain significant amounts of mucin (e.g., mucocele, mucoepidermoid carcinoma) based on prior H&E diagnoses. Cases with inadequate tissue or poor preservation were excluded. A total of 30 suitable tissue blocks were identified. From each block, two serial sections of 3µm thickness were cut using a semi-automatic rotary microtome. Sections were mounted onto glass slides coated with albumin to ensure adhesion during staining procedures[7].

- **Group A (Control)**: Sections stained using the standard Periodic Acid-Schiff (PAS) technique.
- **Group B (Test):** Sections stained using the Kumkum-Schiff technique.

Reagent Preparation:

Periodic Acid Solution (0.5%): Prepared by dissolving 0.5g of periodic acid (H5IO6, ACS Grade) in 100ml of distilled water. Stored in a dark glass bottle at room temperature3.

Schiff Reagent: Obtained as a certified commercial ready-to-use solution. Stored at 4°C in a dark amber bottle.

Kumkum Solution:

Preparation: A standardized solution was prepared using the maceration technique Fifteen grams of commercial *Kumkum* was diluted (Gopuram brand) with 100 mL propan-2-ol. The solutions are kept undisturbed for 48 hours[8]. Filtered solution was used for staining, and the filtrates from those filters were kept in bottles with labels. The solution was then filtered through Whatmann's filter paper No. 1 to remove particulate matter. The filtrate was used as the oxidizing solution. The pH of this solution was checked with a meter and was 6.8. The solution was kept in amber bottles at room temperature away from direct sunlight. It was found that the solution did not change much in color or staining ability for up to three months. To ensure batch-to-batch reproducibility, three independent batches of the staining solution were prepared following the same protocol. Consistency of staining performance was evaluated by comparing color intensity and tissue staining characteristics among the batches, which demonstrated minimal variation.

Pilot study: Prior to the main study, a pilot study was conducted on representative tissue sections to determine the optimal staining duration. Sections were stained for varying intervals of 5, 10, 15, 20, and 30 minutes and subsequently evaluated for staining intensity, contrast, cellular detail, and background staining. Among the tested durations, 15 minutes provided the most consistent and optimal staining characteristics, with clear visualization of tissue architecture and minimal background staining. Therefore, a staining duration of 15 minutes was selected and standardized for all subsequent specimens included in the study.

Staining Protocols:

Group A (Standard PAS):

Deparaffinize sections in xylene 2 changes, 10 minutes each, Hydrate through graded alcohols 100%, 90%, 70% 2 minutes each. Oxidize with Periodic Acid solution for 5 minutes. Rinse in several changes of distilled water. Place in Schiff Reagent for 15 minutes. Wash in running tap water for 5-10 minutes. Counterstain lightly with Hematoxylin and blue the sections. Dehydrate through graded alcohols 70%, 90%, 100% 2 minutes each. Clear in xylene 2 changes, 2 minutes each and mounted with Dibutylphthalate Polystyrene Xylene (DPX)2.

Group B (Kumkum-Schiff):

Deparaffinization & Hydration steps identical to Group A. Treat with freshly prepared *Kumkum* Solution for 15 minutes based on preliminary trials. Rinse in distilled water. Schiff Reaction: Place in Schiff Reagent for 15 minutes. Wash, Counterstain, Dehydration, Clearing and Mounting identical to Group A[2].

Evaluation Criteria: All slides were evaluated independently by two experienced oral pathologists blinded to the staining groups using the scoring system:

1. Cellular architecture (CA):
   Score-0: Indistinct nucleus- cytoplasm
   Score-1: Distinct Nucleus- cytoplasm[1]
2. Quality of Staining (QS)
   Score-0=Poor
   Score-1=Satisfactory
   Score-2=Good[1]

Positive Controls: Sections known to contain mucin (e.g., Mucoepidermoid carcinoma, mucocele) were included in each staining run for both groups to ensure reagent functionality.

3.9. Statistical Analysis: Data were entered into a spreadsheet and analyzed using SPSS software version SPSS 26.0. Inter-observer agreement was assessed using Cohen's Kappa. The Chi-square test was used to compare the distribution of scores for each parameter (CA & Quality of staining) between Group A and Group B. The mean Total Staining Score (TSS) between groups was compared using an Independent Samples T-test (assuming normality). A p-value < 0.05 was considered statistically significant[9].

Results

The 30 tissue blocks represented a spectrum of oral lesions

Table 1: Inter-observer agreement (Cohen's Kappa)

Scoring Category	Cohen's Kappa (k) value	Agreement strength
Cellular architecture	0.85	Very Good
Quality of staining	0.83	Very Good

Table 2a: Cellular Architecture (CA) Preservation Scores

Score	Description	Group A (PAS)	Group B (Kumkum with Schiff)
0	Indistinct Nucleus - Cytoplasm	7	5
1	Distinct Nucleus-Cytoplasm	23	25
Chi square p value		0.003	

Table 2b: Quality of Staining (QS)

Score	Description	Group A (PAS)	Group B (Kumkum with Schiff)
0	Poor	9	10
1	Satisfactory	15	15
2	Good	6	5
Chi square P value		0.002	

Table 3: Total Staining Score (TSS) Comparison

Group	n	Mean TSS ± SD	Range
A (Periodic Acid)	30	14.7 ± 0.2	12-16
B (Kumkum)	30	16.4 ± 0.3	15-18
P value (t test)	0.020		

rich in glycogen or mucin. Cohen's Kappa analysis revealed very good agreement between the two pathologists for all scoring parameters validating the scoring system's reliability. Discrepancies were resolved by consensus review. The data obtained shows Kumkum with Schiff group was statistically superior to Conventional PAS staining in all parameters.

Discussion

This study provides compelling evidence that Kumkum, a naturally derived turmeric-based preparation, serves as an effective and safe oxidizing agent substitute for hazardous periodic acid in the PAS staining technique for oral tissues[10]. Our results robustly support the initial hypothesis: Kumkum solution produced staining results for glycogen and mucin that were statistically significant from the standard periodic acid method in terms of Cellular Architecture preservation and quality of Staining Intensity (p=0.020). The comparable Total Staining Scores confirm the overall equivalence in diagnostic quality.

PAS staining is routinely employed for diagnosing a myriad of oral lesions, from common mucocele to aggressive car-

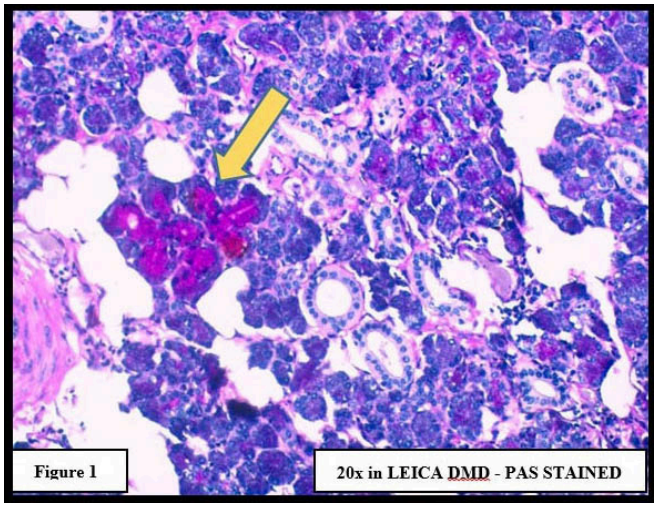

Fig. 1: Photomicrographs showing PAS Staining (Group A) PAS-Schiff: Mucin in salivary glands.

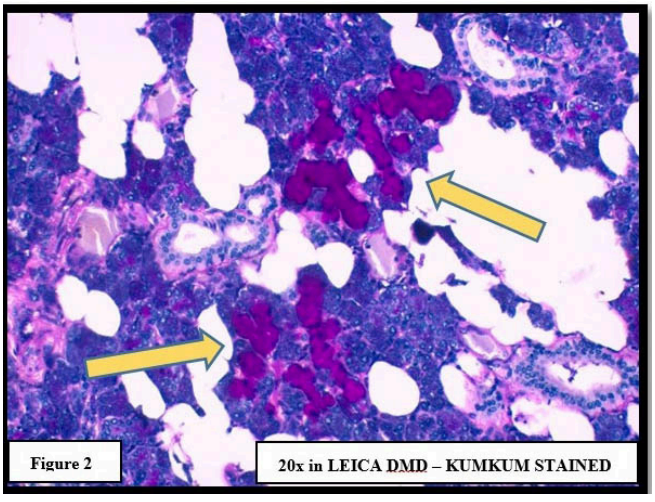

Fig. 2: Photomicrographs showing Kumkum-Schiff Staining (Group B). Kumkum-Schiff: Equivalent mucin staining.

cinomas[11]. The ability to replace periodic acid with Kumkum directly addresses a major occupational health concern. Eliminating a highly flammable and corrosive OSHA Class II chemical from the daily workflow of oral pathology laboratory technicians and pathologists represents a substantial leap forward in laboratory safety. The reduced risk of fire, skin/eye burns, and potential chronic exposure effects cannot be overstated. Handling Kumkum solution requires only standard laboratory precautions (gloves, eye protection), significantly lowering the barrier to safe practice, especially in resource-limited settings or smaller laboratories lacking sophisticated fume hood infrastructure[12].

The observed effectiveness of Kumkum stems from the redox properties of curcuminoids, primarily curcumin. While the exact biochemical mechanism in histochemical oxidation may differ slightly from periodic acid (which acts via cleavage of C-C bonds), the end result – the generation of aldehyde groups from 1,2-glycol moieties – is demonstrably achieved[13]. Our standardized Kumkum solution formulation proved capable of delivering this oxidation step efficiently within a comparable timeframe (15-20 mins vs. periodic acid's 5-10 mins), resulting in robust Schiff reactivity.

The safety advantages of Kumkum extend beyond flammability and corrosivity. Periodic acid requires careful disposal as hazardous waste. Kumkum, being plant-derived and non-toxic, presents a much simpler and environmentally friendlier disposal profile. Furthermore, the potential cost-effectiveness of Kumkum, especially in regions where turmeric is abundant, could make high-quality PAS staining more accessible1,2.

Comparison with Other Natural Alternatives: Our findings position Kumkum favorably compared to other explored natural oxidants. While some plant extracts show promise, Kumkum offers the advantage of being a readily available, culturally familiar substance with inherent staining properties beyond oxidation. Its performance in this study matches the diagnostic gold standard.

Conclusion

This study demonstrates compelling results of Kumkum, a natural turmeric-lime preparation, is a highly effective and significantly safer substitute for periodic acid in the PAS staining technique for oral tissues. It delivers equivalent high-quality visualization of diagnostically critical carbohydrates like glycogen and mucin, preserving cellular architecture and providing intense, specific, and clear staining. Crucially, it eliminates the hazards associated with handling a highly flammable and corrosive chemical, enhancing laboratory safety for Pathologists and laboratory personnel. Kumkum represents a promising, practical, and natural alternative for routine and diagnostic PAS staining in oral histopathology laboratories. Future work should focus on standardization and broader validation.

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