Original Article

Periodic acid–Schiff staining in oral exfoliative cytology of diabetic patients: The odyssey for noninvasive screening – A systematic review and meta-analysis

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ABSTRACT

Background: Diabetes mellitus is a dominant metabolic disorder in the current fast paced culture; its prevalence is escalating worldwide and among all age groups. Guidelines recommend universal screening for diabetes; however, the uptake of these tests in individuals suggests challenges owing to invasive nature of blood collection. Thus, there arises the need for a noninvasive investigation for diabetes screening with a minimum paraphilia and for all medical settings.

Materials and Methods: We have thus conducted a systematic review and meta-analysis according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines, to quantify the association between diabetes and effectiveness of periodic acid–Schiff staining of exfoliative cytology as a screening method. MEDLINE, EMBASE, Cochrane, Scopus, Web of science, CINAHL, and Google Scholar were searched systematically for publications dated till July 20, 2022. Six studies (case–control cross-sectional) were selected and evaluated in depth. The statistical evaluation was done with a forest plot with odds ratio and weightage examined.

Results: It was proved that exfoliative cytology with periodic acid–Schiff (PAS) staining can be used as a screening test for diabetic status evaluation. The findings suggest that the aforesaid noninvasive test is a conclusive screening practice for diabetics.

Conclusion: This systematic review and meta-analysis suggest that PAS staining in exfoliative cytology can be used as a noninvasive screening in diabetic individuals to assess the current level of blood glucose. Given the increased risk of diabetes worldwide, higher quality prospective evidence is suggested in larger sample sizes with other metabolic disorders, ethnicity, and oral disorders to further evaluate the plausibility of the results.

Key Words: Cytology, diabetes mellitus, diagnosis, exfoliative cytology, noninvasive, periodic acid–Schiff staining

INTRODUCTION

Diabetes mellitus (DM) is a global health problem and one of the fastest-growing chronic conditions. It is adjudged that one in two diabetics is unaware of their condition. In the current fast paced and stress fueled culture, this metabolic disorder's prevalence is escalating worldwide and among all age groups.[1] The WHO has predicted that this disorder

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will prevail as the 7th primary cause of fatality by 2030.[2]

For the development of diabetes, the etiological factors are inclusive of obesity, physical inactivity, smoking, and older age. Furthermore, it targets certain ethnic population groups. However, one cause of concern is that it progresses slowly and silently despite microvascular disease resulting in vascular damage during the subclinical phase.[3-5]

Exfoliative cytology, a simple, quick, and inexpensive procedure, is used to study exfoliated desquamated cells. A study by Jajram *et al.* reported the use of exfoliative cytology as a point of differentiation between diabetic and controls. The changes referred to the nuclear area and cytoplasmic area of oral mucosal epithelial cells, N/C ratio, hyperplasia, and an elevation in glycogen deposits in cells of oral epithelium.[6] These reports prompted the authors to use the assessment of this investigation and periodic acid–Schiff (PAS) staining that detects the glycogen deposits as a manner of assessment in diabetes.[7-9]

Diabetes screening is recommended and mandated in health care, enabling an ideal environment for early diabetes diagnosis and management.[4] Considering the tranquil nature of the disorder and the systemic complications, screening tests that are noninvasive and encourage compliance are the need of the hour. The authors also observed that a routine screening for this disorder must be done for all age groups in all medical and dental centers to facilitate early diagnosis, disease control, and minimum of organ damage. The search for an optimum investigation led the way to PAS staining in exfoliative cytology of oral mucosa. With this objective in the forefront, a systematic review and meta-analysis of PAS staining of exfoliative cytology in oral mucosa in cross-sectional observational studies was done. This assessment aimed to derive upon the utility of using this noninvasive methodology as a screening test for diagnosis of diabetes.

METHODOLOGY

Search strategy
The authors systematically searched Scopus, MEDLINE (PubMed), Embase, Google Scholar, Web of Science, CINAHL, and Cochrane Library up to July 20, 2022, for published clinical studies examining the use of PAS staining in exfoliated cells of oral

mucosa of diabetic patients in glycogen assessment [Figure 1]. The systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement guideline with study selection following population, intervention, control, and outcomes criteria.[10,11] The search terms or text terms were: (Periodic acid Schiff staining OR Exfoliative cytology OR oral mucosa OR diabetes mellitus OR Cytomorphometric analysis). The searches were filtered to include only studies written in English, on human subjects. In addition, relevant articles from references were manually searched. Two independent reviewers screened the abstracts and titles for eligibility and to eliminate duplication. The full text of the articles was assessed when the two reviewers ascertained that the abstract or title represented a potentially relevant study. The reviewers critically evaluated the methodology and results of the included studies. The data were extracted independently and discrepancies were adjudicated by consensus.

Eligibility criteria
The studies were screened according to the following inclusion criteria: (a) human subjects; (b) evaluation of PAS staining for exfoliated oral mucosal cells; (c) independent data from the original papers and sufficient information to calculate the outcomes; (d) the population comprised if individuals diagnosed with DM treated with an oral hypoglycemic agent; (e) the selected population was free of additional systemic diseases; and (f) the selected population suffered from medical disorders. Studies were excluded if they (a) were not original articles (e.g., review, case report, or comments); (b) were not human studies (e.g., animal study or *in vitro* study); (c) were duplicate publications; (d) were articles without the necessary information; or (e) were not published in English.

Data abstraction and quality assessment
The data were appraised by one reviewer and the critical evaluation by another reviewer. In the event of disagreements, it was resolved by in depth discussion in the presence of a third reviewer. The Cochrane Handbook for Systematic Reviews-recommended Newcastle–Ottawa Scale (NOS) was referred to assess the selected study quality.[11] The NOS allocates up to 9 points to studies according to three quality domains: selection of the study groups; comparability of the groups; and ascertainment of the outcome.

RESULTS

Characteristics of the included studies

The study search produced 47 studies, of which 6 met with the inclusion criteria. Individual study characteristics are given in Table 1. All the six studies evaluated PAS staining in oral mucosa cells obtained by exfoliative cytology. One of the studies had done PAS staining,[10] while two of the others supplemented with Papanicolaou staining[12,17] and one with PAS diastase staining, respectively.[13] The remaining study additionally estimated the correlation with salivary glucose and blood serum glucose.[14] All the studies were cross-sectional and observational. All the studies were conducted in India, except two which were undertaken in Saudi Arabia and Iran, respectively. The sample sizes ranged from 45 to 150.

Assessment of methodological quality

The core methodological process was quite similar among the studies. All the five studies were rated as high quality (>6 points) and design. The studies comprised high to moderate sample sizes and were conducted in large population-based samples. In addition, the studies had the recorded history of the said medical condition for a minimum time period of 1 year before sample selection. The age was controlled in all the studies, whereas among the gender, it was matched with the controls. The case groups had look back periods with detailed prescriptions and recorded medical histories. In addition, laboratory examinations performed at the time of the study initiation as well.

Screening for diabetes by exfoliative cytology with periodic acid–Schiff staining

A detailed case history and glycemic control reports were evaluated at the start of each of these studies.[7,12-17] At the study outset, a confirmatory test of blood evaluation was conducted in each of the studies. Further, exfoliative cytology was performed on the oral buccal mucosa and devoid of lesions.[16] The collected cells were fixed on a glass slide with alcohol and then stained with PAS reagent. The sensitivity and specificity were noted to be 100% in all the studies,[7,13-17] except one.[12] A forest plot demonstrated the statistical evaluation; PAS staining was effective among case groups,[7,13-17] except in one study.[12]

Graph 1 shows the forest plot of PAS staining of exfoliative cytology from oral mucosa in case and control groups. The line demonstrates that of the six studies included in meta-analysis five studies demonstrate Case groups to be more effective than the Control groups. Thus it was corroborated that the elevated serum glucose level was in correlation with the glycogen demonstrated in staining. The only study in which an exception was noted being that by Asemi-Rad *et al.*[12] where the Case group failed to demonstrate enhanced glycogen storage in diabetic subjects.

The middle line is indicative of the odds ratio (OR) and dot detail entails the 95% Confidence Interval with upper and lower limit. The OR is the strength of association between the exposure and the outcome. The OR and the weightage highlighted the study by Salih *et al.*[17] to have a strong relationship than the
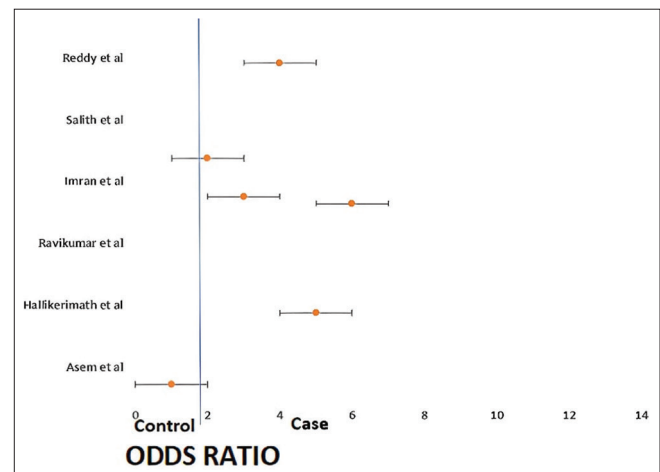
Graph 1: Forest plot of PAS staining of exfoliative cytology. PAS: Periodic acid–Schiff.

Table 1: Aggregate data of selected studies

| Author	Year	Country	Study design	Single/multicenter	Financial COI	Sample size
S S Ravikumar *et al.*	2019	India	Prospective	Single	Nil	45
Imran.A *et al.*	2019	India	Prospective	Single	Nil	150
Salih. M. M *et al.*	2018	Saudi Arabia	Prospective	Single		90
Asemi-Rad *et al.*	2013	Iran	Prospective	Single	Nil	100
Hallikermath *et al.*	2011	India	Prospective	Single	Nil	60
Reddy *et al.*	2018	India	Prospective	Single	Nil	60

COI: Contribution
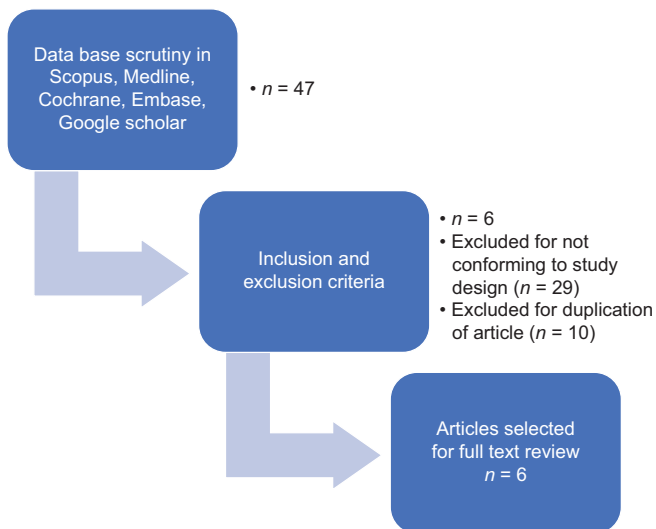
Figure 1: Flowchart on selection strategy.

other studies. In addition, Salih *et al.*'s[17] study had a highest weightage of the selected studies in terms of association of the serum glucose level and glycogen staining in the selected study sample.

DISCUSSION

This systematic review and meta-analysis of six studies suggested that PAS staining in exfoliative cytology is recommended for screening in diabetic patients. The findings were particularly favorable for diabetic screening, with low heterogeneity across studies.

To our knowledge, this is the first systematic review and meta-analysis, evaluating the use of PAS staining techniques for diabetic screening. The robustness of this study includes quantifying the specific correlation between the glycogen storage in oral mucosal cells and the serum blood sugar and thus the staining methodology is corroborative for the evaluation. Compared with prior research, our review resulted in a pool of eligible studies for the purpose of comparison, as no reviews were available on the subject during the course of this manuscript writing. In addition, the methodology quality was high, no loss to follow–up, or nonresponse bias among the selected studies.

It is brought to attention that this systematic review focused on studies reported in English alone. However, as per a prior comprehensive review by evidence synthesis experts,[18-20] restrictions in language do not influence systematic biases that could affect the result interpretation.[21-23]

The main advantages of this diagnostic methodology were the noninvasive, relatively painless, inexpensive, and require a minimum of technical skill. This was in accordance with the findings by Latti *et al.*[24] Studies by Ahmed *et al.* and Acha *et al.* attest to the use of the oral cytologic analysis as a useful early diagnostic method.[25,26] The use of PAS staining in the selected studies maintain that the cells in all the study groups displayed binucleation, decreased cytoplasmic/nuclear ratio, nuclear enlargement, enucleation and inflammation as compared to the control groups. This was in tandem with the reporting of Shareef *et al.* and Alberti *et al.* It was further affirmed that DM alters the oral epithelial cells, detectable by microscopy and cytomorphometry.[27,28] The limitations of this methodology from the author standpoint was that the glycogen deposits indicative of diabetes could not be quantified or standardized unlike serum blood glucose. In the same vein, this diagnostic methodology is not indicative of long-term glucose control evaluation, attributed to the increased turnover of oral epithelial cells.

CONCLUSION

This systematic review and meta-analysis suggest that PAS staining in exfoliative cytology can be used as a noninvasive screening in diabetic individuals to assess the current level of blood glucose. The procedure demonstrated active patient compliance owing the ease of procedure and because it can be performed both in a medical as well as dental clinics. Caution is warranted in interpreting these findings due to the fact that this investigative method does not indicate long-term glucose levels. Given the increased risk of diabetics in this population, future studies should consider using higher population quantity and diversity to assess the quality of this investigative method.

Ethical statement
The study did not require consent from patients or from the institution.

Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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