NEXT GENERATION BASED MICRORNA SEQUENCING IN ORAL SQUAMOUS CELL CARCINOMA- A SYSTEMATIC REVIEW

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Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

Background: Next-generation sequencing (NGS) is a powerful tool that has enabled the systematic study of genomes and provided researchers with insights into disease understanding. NGS is a massive and parallel DNA sequencing technology for large-scale, ultra-high throughput, and automated high speed genome analyses.NGS has a wide variety of applications for the study of biological systems at a new level.NGS-applied OSCC research has also identified various genetic alterations and detected mutations with low variant allele frequency.
Aim: This paper aims to systematically review next generation based microRNA sequencing in Oral Squamous cell carcinoma
Methods: A search was done using MeSH terms and keyword search in the electronic databases namely PubMed, Google Scholar, Cochrane, Science Direct ,Lilacs and addition searches were carried out through cross checking the bibliographies of selected articles. Then based on the inclusion and exclusion criteria and availability of the full texts, a total of 2 articles were included in this systematic review
Result: The search yielded a total of articles out of which 56 articles were included based on the eligibility criteria. Quality assessment based on the Quality Assessment of Diagnostic Accuracy Studies 2 tool was used to obtain a risk of bias chart using Revman 5.4 software and it was proved that from the 2 included studies, one had low risk of bias and another had moderate risk of bias.
Conclusion: This systematic review aimed at improving the current understanding of next generation sequencing and applicability in OSCC. Further, this may be an alleyway for the identification of newer biomarkers using NGS-based technique.

Keywords: Next Generation, Sequencing, Biomarker, Oral Squamous Cell Carcinoma

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DOI: 10.31838/ecb/2023.12.s2.151

1. Introduction

Oral Squamous Cell Carcinoma(OSCC) is the most common malignant epithelial neoplasm affecting the oral cavity.OSCC accounts for 90%- 96% incidence of whole head and neck cancers and the Survival rate is about 50% of affected cases. Prediction of survival in oral cancer depends on classical parameters such as tumor grade and depth of invasion.Although many biomarkers have been introduced as potential prognosticators of OSCC,there are no sensitive biomarkers for detection of OSCC.(1)(2)

Biomarkers found in the saliva are an ideal non-invasive diagnostic tool for early diagnosis of cancer.NGS is a massive and parallel DNA sequencing technology for large-scale, ultra-high throughput, and automated high-speed genome analyses. NGS helps in determining the order of nucleotides in entire genomes or targeted regions of DNA or RNA and has revolutionized the biological sciences. NGS has a wide variety of applications for the study of biological systems at a new level. NGS can be used to sequence entire genomes or specific areas of interest.(3)NGS-applied OSCC research has also identified various genetic alterations and detected mutations with low variant allele frequency.(4) NGS is also becoming an essential method in characterization of salivary gland tumors.(1) However, NGS has only rarely been applied to the identification of salivary biomarkers of OSCC.The main aim of this systematic review is to explore the expression of microRNAs through next generation sequencing in oral squamous cell carcinoma

2. Materials and methods

Study design & search methodology

For this study, we followed the guidelines given by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Guidelines (PRISMA).

The studies included in this systematic review were identified by a comprehensive search from the following search engines using the keywords.PubMed Advanced search using MeSH terms ,Google scholar,Cochrane ,ScienceDirect,Latin American and Caribbean Health Sciences Literature (LILACS) upto Hand searching of relevant articles was done until September 2022 [Figure 01]

Search strategy

For the search strategy, Mesh terms and free text words were combined through Boolean operators: (((Next generation sequencing OR sequencing of microrna OR miRNA OR microrna))) AND ((OSCC OR 'oral squamous cell carcinoma' OR 'oral cancer' OR 'Tongue Cancer' OR TSCC OR OTSCC OR Gingiva OR 'Head Neck'))

Eligibility criteria

Inclusion criteria

1.Studies written in the English language
2.Proven diagnosis of OSCC by histopathology
3.Studies performed using next generation sequencing

Exclusion criteria

1.Animal studies were excluded
2.Studies done on other carcinoma were excluded
3.Abstract only

Selection process

Two investigators independently evaluated articles retrieved from the databases. First round of evaluation was performed by reading only the title and abstracts of the studies. At the end of the first round all studies considered eligible were included for full-text evaluation. A direct search of bibliographies of articles in full-text wascarried out, to find outfurther articles to include. After full-text reading, only studies considered eligible by both authors were included.

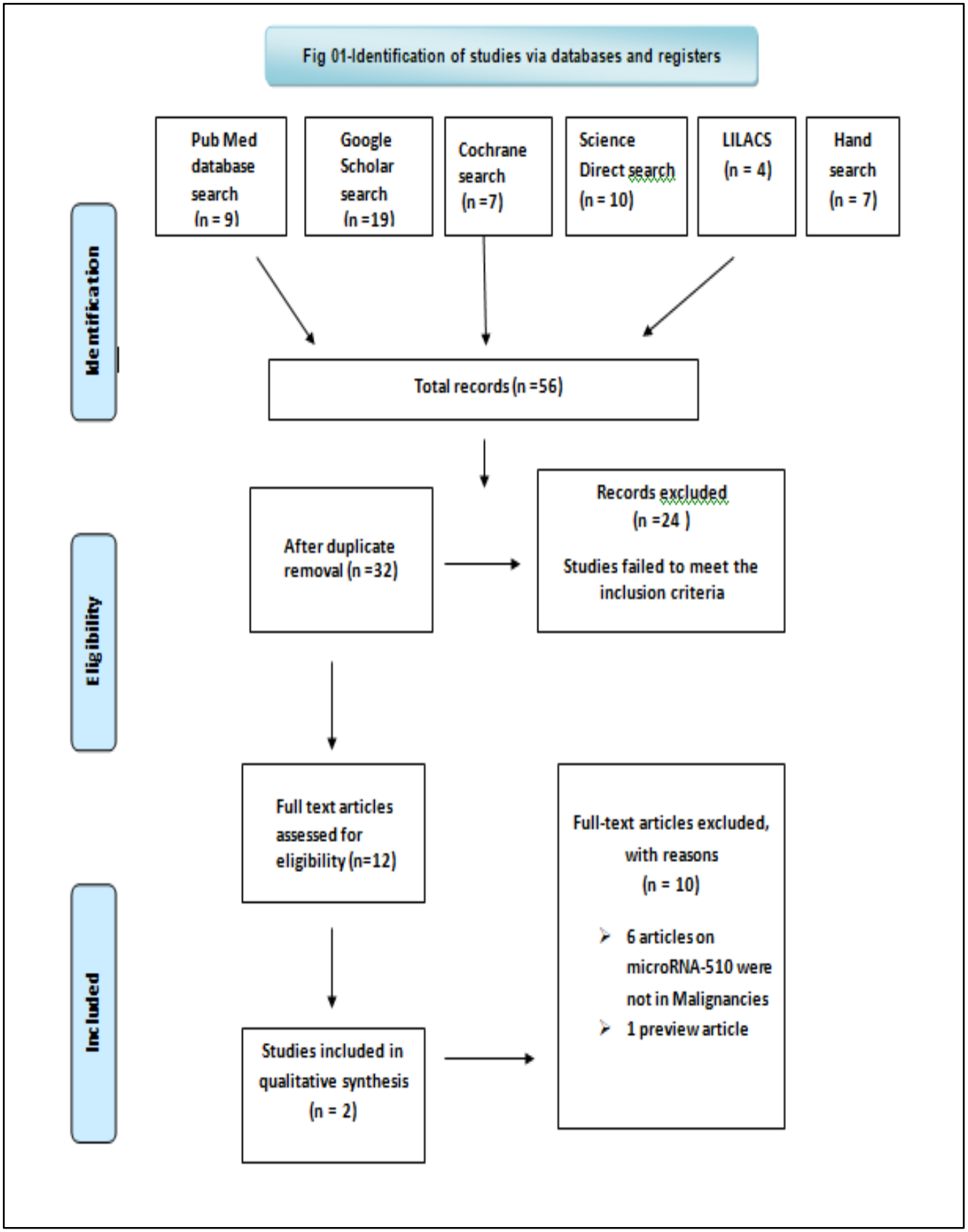
Fig 01-Identification of studies via databases and registers
Identification
Pub Med database search (n = 9)
Google Scholar search (n =19)
Cochrane search (n =7)
Science Direct search (n = 10)
LILACS (n = 4)
Hand search (n = 7)
Total records (n =56)
Eligibility
After duplicate removal (n =32)
Records excluded (n =24)
Studies failed to meet the inclusion criteria
Included
Full text articles assessed for eligibility (n=12)
Full-text articles excluded, with reasons (n = 10)
6 articles on microRNA-510 were not in Malignancies
1 preview article
Studies included in qualitative synthesis (n = 2)

Data extraction

Data extraction of the characteristics of included studies and the variables of outcome are given in (Table 01)

Author/Year	No.of samples	Site	Sample type	Stage	Result
Siavosh et al 2017	5 samples	3-primary tumor site 1-Lymph node metastasis 1-Blood	Fresh frozen sample	Stage III OSCC	24 mutations identified in the recurrence biopsy,14 mutations shared by primary tumor
Hui-Hwa et al 2017	2 samples	Tissue biopsies from buccal mucosa and tongue	Fresh frozen sample	Not mentioned	45 miRNAs upregulated 17 miRNAs downregulated

Table 01- Data extraction and characteristics of the Included studies

Quality assessment of the studies

The quality of these 9 studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool. This tool includes 14 items which assess the risk of bias and sources of variation in diagnostic studies. It is recommended by the Cochrane Collaboration, Agency for Health Care Research and Quality and the UK National Institute of Health and Clinical Excellence to assess the quality of diagnostic studies. QUADAS-2 is an improvised redesigned tool from the Cochrane Collaboration based on feedback from editors of the original QUADAS tool

Risk of bias

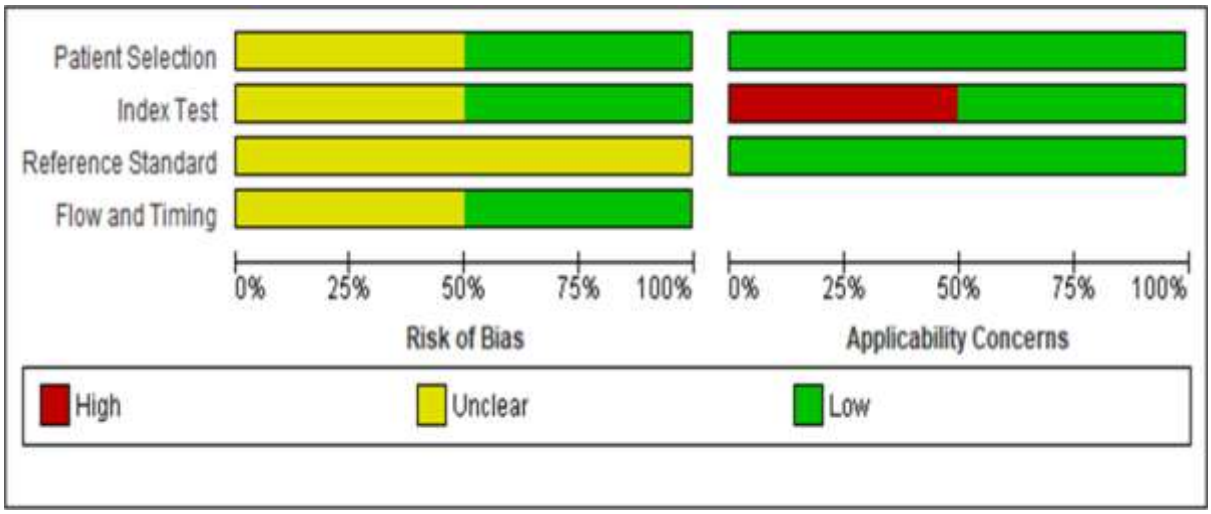

Figure 2 – Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

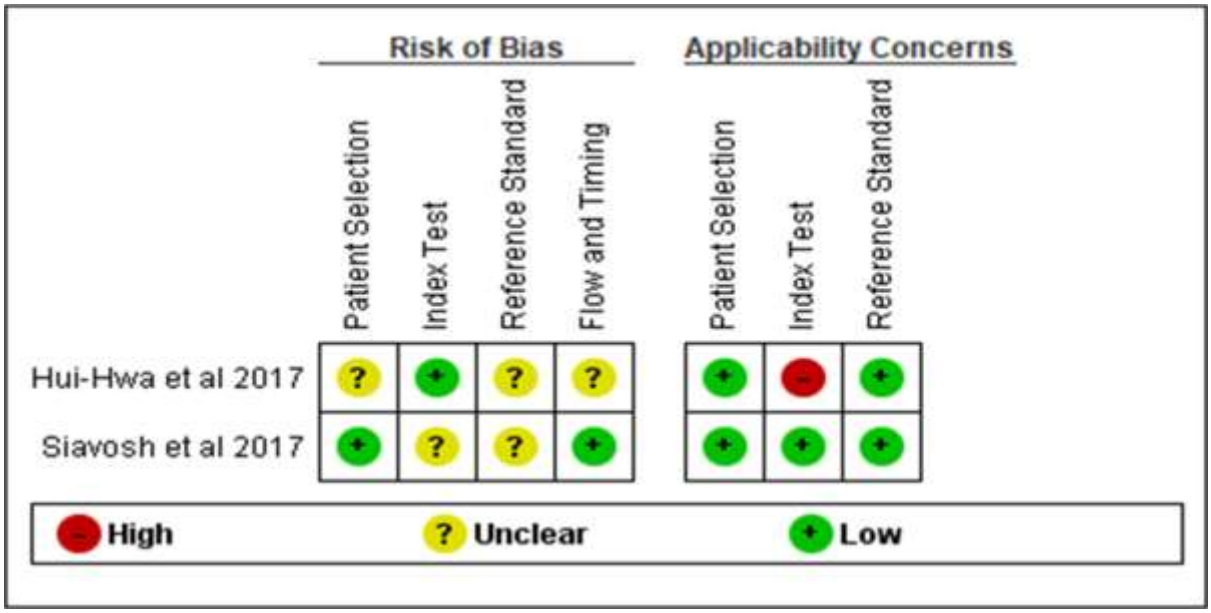

Figure 3 – Risk of bias graph: review authors' judgements about each risk of bias item across all included studies.

3. Result

Risk of bias assessment was performed using RevMan 5.4.Among the 2 studies,
study by Siavosh et al showed moderate risk of bias, study by Hui-Hwa et al 2017 showed low risk of bias.Siavosh et al identified 24 mutations in the recurrence biopsy and 14 mutations are shared by the primary tumor.This mutation indicates pre-neoplastic field or primary tumor evolution.Study by Hui-Hwa et al had more than 10 million clean reads in each library.In this study of OSCC tissues, 73 and 24 miRNAs were up-regulated and down-regulated, respectively, in patient 1. 54 and 25 miRNAs were up-regulated and down-regulated, respectively, in patient 2.These combined profiles revealed that a total of 62 miRNAs are significantly up regulated (n=45) or down regulated (n=17) in the two NT-paired samples

4. Discussion

This systematic review aimed to summarize the sequencing of microRNA in oral squamous cell carcinoma.NGS-based research has been used to identify factors in metastasis of lung, prostate, ovarian, and bile duct cancer. NGS-based evaluation of candidate biomarkers in saliva could also be routinely used as a simple, non-invasive test in patients with OSCC.(1)(5)NGS has also been used as a valuable tool for detecting novel biomarkers in periodontal disease.(3,6)Current applications of NGS in cancer research and to propose potential genomic and proteomic saliva biomarkers for NGS-based study in OSCC screening and diagnosis programs is enlightening.(7) For the establishment of a standardized research and designed protocol for the detection of trace protein or nucleic acids biomarkers from saliva samples, efficient and stable collection, processing and preservation methods should first be confirmed.(8)Buyanbileg et al.,concluded that the selection of potential OSCC biomarkers through NGS-based protein studies from epithelial tissue cells in collected saliva will be possible. These non-invasive methods could be a useful tool for the improvement of an immune biosensor for ensuring general public health and for diagnosis of OSCC before its metastasis or progressive infiltration to adjacent tissues. Based on NGS analysis, we will be able to verify the biomarkers of OSCC in saliva and to effect early diagnosis of oral cancer based on this.(1)
Hui-Hwa Tseng et al.,compared the miRNA profiles of OSCC tissues and corresponding adjacent normal tissues in two patients with OSCC. In both, the expression of oncogenic miR-21-5p and -3p, that were generated from the same precursor (pre-mir-21), was up-regulated in the OSCC tissues. The expression of the guide strand, miR-21-5p, which is up-regulated in human cancers, has been well-studied in most cancerous tissues and has been reported to play an oncogenic role in regulating cancer cell proliferation, cell cycle, invasion, and drug resistance.(9)(10)
Soyeon Kim et al.,discussed the frequently identified gene alterations and miRNAs that are associated with the development and progression of OSCC. Although many studies have confirmed various gene mutations and miRNAs related to OSCC, more research is necessary for a deeper understanding of the molecular processes involved in tumorigenesis. In addition, NGS plays a crucial role in novel discoveries, but its clinical

capabilities are not yet being fully applied. Numerous studies have used qRT-PCR or microarray to confirm the existence of known miRNAs. However, only a few studies have discovered novel miRNAs using NGS despite the introduction of NGS over a decade ago. To highlight the importance of NGS application in OSCC studies, this paper not only discusses the use of NGS in identifying a malignancy, but also implies the need for further research using this technique. Interesting research questions can be derived from studies discussed in this review. Several papers have mentioned the possibility of particular gene alterations appearing in higher proportions in certain ethnicities. It will be important to identify any ethnicity-associated mutations to optimize OSCC prevention and treatment. A previous study has also suggested the increasing prevalence of OSCC in younger patients. These reasons emphasize the need for more research regarding OSCC-related miRNAs using a high-throughput method for accurate and efficient sequencing.(7,11).Our team has extensive knowledge and research experience that has translate into high quality publications (12–21))

5. Conclusion

Like many cancers, early-stage OSCC is difficult to detect. A significant number of patients do not seek clinical care until the OSCC is in an advanced stage. The development and advancement of screening and early diagnosis approaches has been recommended as the most effective strategy for reducing the OSCC-related morbidity and mortality rate. However, NGS has only rarely been applied to the identification of salivary biomarkers of OSCC

Pico analysis
P (population) - Oral Squamous cell carcinoma
I (intervention)-microRNA
C (comparison)-Nil
O (outcome)-Sequencing

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