SYSTEMATIC REVIEW

MicroRNAs obtained from cervical swabs in predicting preterm birth

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ABSTRACT

Objective: Identifying the risk of preterm birth is crucial for early intervention. miRNAs, small non-coding RNAs that regulate gene expression, play a key role in development and tissue maintenance. Under stress conditions, their regulatory functions become significant, linking them to disease states. Using miRNAs from cervical swabs as potential biomarkers could revolutionize preterm birth risk assessment. This systematic review examines current research on the effectiveness of cervical swab miRNAs in predicting and estimating preterm birth risks, aiming to enhance early detection and management strategies for preterm births.

Materials and Methods: Using PubMed database, 14 articles were obtained using the keywords "microRNA" and "preterm". Reviews and unrelated studies were then excluded from both pooled articles, resulting in 4 articles included in the final review. Risk of bias were examined using the Newcastle Ottawa Scale. Sample characteristics, time of cervical swab collection, and results from each study were extracted for further analysis.

Results: A total of 4 articles were included in this review. Various miRNAs were examined in and were generally successful in predicting preterm birth. miRNA-145, miRNA-199, miRNA-30, miRNA-21, and miRNA-181 family were examined by multiple studies and produced significant results in predicting preterm birth. Based on enrichment analysis, various miRNAs were found to be involved in several biomolecular signaling pathways leading to preterm birth, such as inflammation, chemokine and cytokine signaling pathway, and toll-like receptor signaling.

Conclusion: miRNAs obtained from cervical swabs exhibit statistically significant difference in expression between women with term births and preterm births. Further studies are needed to improve the predicting power and accuracy of miRNAs in preterm births.

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Highlights:

1. Increased expression of certain miRNAs in women experiencing preterm birth could be linked to various molecular pathways which contributes to preterm birth.
2. miRNAs obtained from cervical swabs exhibit statistically significant difference in expression between women with term births and preterm births.

INTRODUCTION

Preterm birth is common and frequently encountered problem in the world. A total of 13.4 million babies were estimated to be born preterm, defined as delivery before 37 weeks of gestation, in 2020.[1,2] Preterm birth is affected by various etiologies, such as individual and environmental factors.[3,4] Some of the known risk factor for preterm birth are adolescent pregnancies, advanced maternal age, low maternal education, and history of previous preterm birth.[5,6] Preterm birth is also accompanied by various related morbidities, such as cerebral palsy, bronchopulmonary dysplasia, and retinopathy of prematurity.[7] The consequences of preterm birth also persist until early adulthood, causing worse neurodevelopmental outcomes, higher rates of hospital admissions, and learning difficulties.[8,9] Identification of possible risk of preterm birth in pregnant woman bears a lot of importance in preparing and taking early action against preterm birth.[10,11]

Clinical studies show that a short cervix is one of the most reliable indicators of preterm delivery.[5,6] However, the molecular equivalent of a sonographic short cervix is still unclear. Indeed, while a sonographic short cervix is thought to be a proxy for "premature cervical remodeling," there is a dearth of information showing what is truly happening (molecularly, biochemically) in cervical tissue at the time a sonographic short cervix is detected. This knowledge is crucial to developing more effective preterm birth prevention techniques. It is well understood that cervix remodeling occurs at any gestational age in order for the fetus to pass through. Investigations on cervical biopsies of women at term showed that gene expression patterns in the cervix differed between laboring and nonlaboring women, as well as between women with and without a ripe cervix. Although these studies shed some light on the process of cervical ripening at term, they use a more intrusive way to determine the molecular phenotype of the pregnant cervix. Notably, no studies have been conducted so far that give information on the molecular changes that occur in the cervix and may be connected with early cervical remodeling and preterm parturition.[12]

miRNAs are tiny, noncoding, single-stranded RNAs that average 22 nucleotides long. miRNAs control gene expression by binding to messenger RNA (mRNA) and repressing it, but they may also trigger translation and regulate transcription.[13] Most species have fewer miRNAs (estimated at around 1000 in the human genome) than mRNAs (roughly 30,000). However, a single miRNA may influence hundreds of mRNAs, resulting in profound and far-reaching impacts on gene expression networks. miRNAs appear to play a fundamental function in controlling development and tissue homeostasis through specialized regulation or "fine-tuning" of gene expression. However, in specific situations (e.g., stress, inflammation, hypoxia), the functions of miRNAs become evident, suggesting that miRNAs play a vital role in disease states.[12] The use of miRNAs obtained from cervical swabs to predict possible risk of preterm birth could be revolutionary in the management of preterm birth. The properties of miRNAs, such as its stability in room temperature and changes in pH, make them ideal biomarkers.[14]

This systematic review aimed to summarize the current findings of researches regarding the plausibility and effectivity of miRNAs obtained from cervical swabs to predict and estimate the risk of possible preterm birth.

MATERIALS AND METHODS

Search strategy

The keywords used in the search were “microRNA” and “preterm”. The results following the search were reviewed by the reviewer to determine the eligibility of the study. This study was conducted according to the PRISMA guideline.[15]

Inclusion criteria

The inclusion criteria for the study encompassed several key aspects. First, the study must be published in English. Second, it should specifically focus on investigating the association between microRNAs extracted from cervical swabs and preterm birth. Third, the publication date of the study must fall within the timeframe from the year 2000 onwards.

Exclusion criteria

The exclusion criteria for the study delineated specific conditions that rendered studies ineligible for inclusion. First, studies not published in English are excluded from consideration. Second, studies conducted in vitro are also excluded from the scope of inclusion. Additionally, studies exploring microRNA obtained from sources other than cervical swabs are excluded. Finally, studies published prior to the year 2000 are not considered within the defined parameters.

Selection process

Utilizing the PubMed database, 14 articles were identified and screened for eligibility. One review article was excluded, leaving 13 articles left for full-text

retrieval. All 13 articles were available for retrieval and were further assessed for eligibility for the final review. A total of 9 articles were excluded from the 13 articles assessed. The reason for exclusion were : studies done in animals (n = 2), studies with HIV patients as population (n = 1), miRNAs examined from blood plasm (n = 1), no cervical swabs examined as object of interest (n = 2), studies examining other types of RNA (n = 1), and studies examining other markers (n = 2). The final review included a total of 4 articles that fulfilled the criteria described beforehand. Article selection process described in Figure 1.

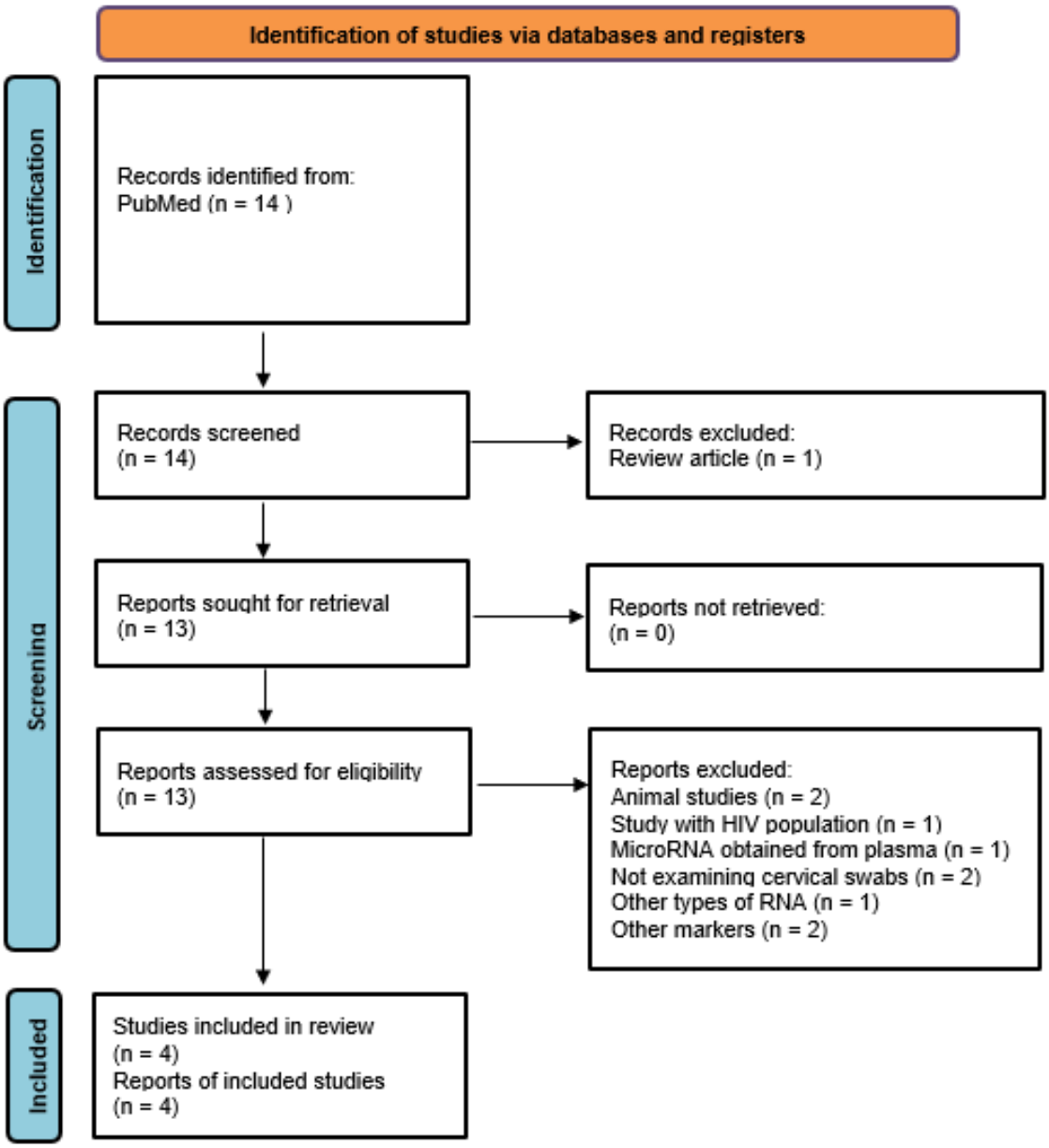

Figure 1. Article selection process according to PRISMA flowchart.[15]

Risk of bias assessment

Risk of bias assessment in animal studies were done using the Newcastle Ottawa Scale for case-control and cohort studies. The Newcastle Ottawa Scale examines three components: selection, comparability, and outcome. The selection components were examined using four indicators: representativeness of the exposed cohort, selection of the non-exposed cohort, ascertainment of exposure, and demonstration that outcome of interest was not present at the start of the study. The comparability component was examined by reviewing the comparability of cohorts based on the design and analysis. Lastly, the outcome component was assessed by using three indicators: assessment of outcome, whether the follow-up was long enough for outcomes to occur, and adequacy of follow up of cohorts.[16] Risk of bias assessment is described in Table 1.

Table 1. Risk of bias assessment

	Selection	Comparability	Outcome
Burris et al., 2023	****	**	***
Sanders et al., 2015	****	*	***
Peng et al., 2020	****	*	***
Elovitz et al., 2014	****	*	****

Data Extraction

The following data were extracted from the studies including: author name, year of publication, study participants, time of cervical swab collection, and results. Extracted data are described in Table 2.

Table 2. Study characteristics

First Author	Year	Design	Participants	Collection of cervical swab	Results
Burris	2023	Case-control	sPTB : 25, control : 49, subjects were matched by age, parity, and race	Mean gestational age of 17.1 weeks (4.8 months) of gestation	Cervical microRNA expression was significantly higher in the sPTB group compared to control group. A total of 95 individual microRNAs were significantly upregulated (>2-fold change) in the sPTB group compared to control group.
Sanders	2015	Prospective cohort	53 pregnant Mexican women	Between 16 and 19 weeks of gestation	A total of 6 miRNAs were significantly associated with gestational age at the time of delivery (miR-21, miR-30e, miR-142, miR-148b, miR-29b, miR-223). Gestations were 0.9 days shorter on average for each doubling in miR-21 expression and by 1-1.6 days shorter on average for each doubling of miR-30e, miR-142, miR-148b, miR-29b, miR-223.
Peng	2020	Case-control	PTB : 52, PPROM : 60, term birth : 70	Within 30 minutes of delivery	A significant decrease in miR-199a-3p in both the PTB and PPROM groups compared to term birth group.
Elovitz	2014	Case-control	PTB : 10, term birth : 10	Between 20 weeks to 23 weeks 6 days gestation and between 24 weeks to 27 weeks 6 days gestation	A total of 99 miRNAs differed between the PTB and term birth group and 24 miRNAs had a >2-fold change between the PTB and term birth group.

Table 3. Significant miRNAs analyzed in the studies

	First Author			
	Burris	Elovitz	Sanders	Peng
Significant miRNAs mentioned by multiple studies	145	145	-	-
	199b	199a-3p / 199b-3p 199b-5p	-	199a-3p
	30a-5p 30d 30e.3p	30e	30e-5p	-
	29a	-	29b-3p	-
	21	-	21	-
	181c	181a-1 / 181a-2	-	-
Significant miRNAs mentioned by only one study	28.3p	140-5p	142-3p	-
	23b	338-5p	148b-3p	-
	542.3p	19a	223-3p	-
	29b	148b	-	-
	130b	106b	-	-
	28	128-1 / 128-2	-	-
	425.5p	3176	-	-
	9	374b	-	-
	342-3p	.24-02	-	-
	1291	15a	-	-
	7	25	-	-
	942	1260	-	-
	-	143	-	-

RESULTS AND DISCUSSION

A total of four articles were included in the final review.[12,13,17,18] Risk of bias assessment utilizing the Newcastle Ottawa Scale resulted in no significant risk of bias in the articles included. A summary of study characteristics could be found in Table 1 and 2.

Studies authored by Burris et al. and Elovitz et al. both produced significant results for miR-145. Burris et al. reported a fold change of 3.2 (OR : 1.71, 95% CI : 1.21-2.40, p = 0.0021) and Elovitz et al. reported a fold change of 11.5 (p = 0.0009).[12,13] The miR-199 family was found to be significant by Burris et al. (miR-199b, fold change : 6.7, OR : 1.48, 95% CI : 1.09-2.00, p = 0.012), Elovitz et al. (miR-199a-3p/miR-199b-3p, fold change : 7.2, p = 0.0048 and miR-199b-5p, fold change : 2.4, p = 0.0041), and Peng et al. (p < 0.001).[12,13,17] The miR-30 family was found to be significant by Burris et al. (miR-30a-5p, fold change : 2.2, OR : 1.9, 95% CI : 1.25-2.88, p = 0.0027, miR-30d, fold change : 2.1, OR : 1.95, 95% CI : 1.25-3.05, p = 0.0035, and miR-30e.3p, fold change : 2.6, OR : 1.47, 95% CI : 1.11-1.95, p = 0.0078), Elovitz et al. (miR-30e, fold change : 2.9, p = 0.0047), and Sanders et al. (miR-30e-5p, p = 0.016).[12,13,18] The miR-29 family was found to be significant by Burris et al. (miR-29a, fold change : 3.9, OR : 1.62, 95% CI : 1.17-2.24, p = 0.0039) and Sanders et al. (miR-29b-3p, p = 0.045).[13,18] miR-21 was found to be significant by Burris et al. (fold change : 2.6, OR : 1.48, 95% CI : 1.10-1.98, p = 0.0091) and Sanders et al. (p = 0.009).[13,18] The miR-181 family was found to be significant by Burris et al. (miR-181c, fold change : 3.1, OR : 1.37, 95% CI : 1.07-1.74, p = 0.0115) and Elovitz et al. (miR-181a-1/ miR-181a-2, fold change : 3.81, p = 0.0048).[12,13] A summary of the miRNAs mentioned in the studies could be found in Table 3.

Utilizing data obtained in their study, Burris et al. performed an AUROC calculation, resulting in a value of 0.71, successfully predicting sPTB 71% of the time.[13] miRNA enrichment analysis also shown several pathways that could contribute in PTB (inflammation, chemokine and cytokine signaling pathway (IL-4 and IL-1), and Toll-like receptor signaling).[13,19,20] Peng et al. in the investigation of miR-199a-3p, discovered a link between miR-199a-3p and the HMGB1 signaling in the inflammation pathway.[17] miR-199a-3p inhibits the expression of HMGB1, which activates TLR4, leading to NF-kB activation, inducing the production of pro-inflammatory cytokines (IL-1β, TNF-α, and others).[17]

Results from multiple studies suggests that the increased expression of certain miRNAs in women experiencing preterm birth could be linked to various molecular pathways which contributes to preterm birth. The miRNAs found to be significant in multiple studies could be utilized as a ground for further research regarding those specific miRNAs. An AUROC value of 71% obtained by Burris et al. suggests the potential of miRNAs obtained from cervical swabs in predicting and estimating the risk of preterm birth in pregnant woman.[13] Further studies with a larger sample size, performed in more genetically diverse participants, and

implementing AUROC calculations could pave the way for future research and implementations on this topic.

The strength of this systematic review lies in its topic novelty. Research in the use of miRNAs obtained from cervical swabs in predicting preterm birth is a rather new and novel concept, being only examined by a selective few studies. Our summarized findings from multiple studies could lead to a development of further studies examining the applications of miRNAs obtained from cervical samples to predict future preterm birth with emphasis on miRNAs found to be significant and appearing in multiple studies such as the miRNA-145, miRNA-199, miRNA-30, miRNA-21, and miRNA-181 family. Future research could draw inspiration from this finding and focus on combining these miRNA families into a more definitive and comprehensive sampling and examination, resulting in a more detailed and powerful predictive value. The limitations of this study are its lack of available material, again, due to its novelty in topic. Further research is highly encouraged in this topic due to its proven significance, proved by multiple studies. Further studies down the line could discover many more miRNAs that could possibly contribute in the development of preterm birth. The studies included in this systematic review are mostly case controls, with only one cohort study identified in the literature searching process. Generally, case control studies suffer from multiple limitations, such as recall bias and selection bias. The use of the Newcastle Ottawa Scale risk of bias assessment tool helped to mitigate any possible potential for bias, but cannot eliminate completely the disadvantages of a study design. A cohort study design is preferable due to its strength of establishing the causality between events, in this case between miRNAs and the event of preterm birth. However, due to the nature of cohort designs requiring more time and resources, this study design is harder to execute in actual research setting. Another limitation of the study is the various time point where cervical swabs are collected, complicating the comparison process between studies and its results. A future study could be done in collecting cervical swabs in multiple time points in one pregnant woman in order to discover the optimal point of cervical swabs sampling with emphasis on the fold change expressed by various miRNAs in various time points. Such study could be done in conjunction with the antenatal care service provided by obstetricians in a regular clinical setting. The sample characteristics displayed in multiple studies also makes it rather difficult to apply the findings to the general population, necessitating a need for research in detecting which miRNAs are most prominent in each population base.

CONCLUSION

miRNAs obtained from cervical swabs exhibit statistically significant difference in expression between women with term births and preterm births. Further studies are needed to improve the predicting power and accuracy of miRNAs in preterm births.

DISCLOSURES

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Conflict of interest

There has no conflict of interest.

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Author contribution

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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