Research Article

Dermatology Research

Serum Micronutrient Levels In Premature Canities: A Systematic Review and Meta-analysis

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ABSTRACT

Background: *Premature canities, also known as premature graying of hair, is the graying of hair before 20 years old in Caucasians, before 25 years old in Asians, and before 30 years old in African–Americans. The risk factors for premature canities are multifactorial. The role of micronutrient deficiency has long been suggested to be involved in the etiopathogenesis of premature canities. The management of premature canities remains limited and far from satisfactory; hence, knowing what vitamins and minerals can help in treating premature canities is vital.*

Aim: *This study aims to analyze serum vitamin B12, iron, ferritin, copper, calcium, and zinc levels in premature canities.*

Methods: *The MEDLINE/PubMed electronic database, Cochrane library, ClinicalTrials.gov, EBSCO, Scopus, ProQuest, Cambridge Core, reference lists, conference proceedings, and researchers in fields of eligible studies were searched. Twelve studies (n = 1791 subjects) were included in qualitative analysis of which nine studies (n = 1381 subjects) were included in meta-analysis. Serum levels of micronutrients (vitamin B12, iron, ferritin, copper, calcium, and zinc) were compared in the premature canities and nonpremature canities groups.*

Result: *Pooling of data using fixed-effects model found the overall effect mean difference serum vitamin B12 level was −0.697 ± 0.105 (95% CI = −0.904 to −0.490), p < 0.001. The overall mean difference serum iron level was 0.146 ± 0.105 (95% CI = −0.058 to 0.350), p = 0.161. The overall mean difference serum ferritin level was 0.720 ± 0.071 (95% CI = 0.585 to 0.854), p < 0.001. The overall mean difference serum copper level was 0.230 ± 0.118 (95% CI = −0.003 to 0.463, p = 0.053. The overall mean difference serum calcium level was −0.443 ± 0.114 (95% CI = −0.655 to −0.212), p < 0.001. The overall mean difference serum zinc level was −0.104 ± 0.138 (95% CI = 0.377 to 0.170, p = 0.457. Qualitative analysis showed that vitamin B12 plays an important role in DNA synthesis in the proliferation of hair follicle cells and in melanogenesis. Iron, ferritin, copper, calcium, and zinc also play important roles in stabilizing and increasing tyrosinase activity in several stages of the melanogenesis process.*

Conclusion: *The meta-analysis showed that serum levels of vitamin B12, ferritin, and calcium were significantly lower in premature canities. Although the lower serum iron, copper, and zinc levels were not significantly different in premature canities compared with nonpremature canities. Qualitative analysis found that deficiency of vitamin B12, iron, ferritin, zinc, copper, and calcium can affect the physiology of hair melanogenesis that causing premature canities.*

Keywords
Premature canities, Micronutrients, Vitamin B12, Iron, Ferritin, Calcium, Copper, Zinc.

Abbreviations
CI: Confidence interval; PRISMA: Preferred Reporting Items of Systematic Reviews and Meta-Analyzes; STROBE: Strengthening the Reporting of Observational Studies in Epidemiology; TRP-1: Tyrosine-related Protein-1; TRP-2: Tyrosine-related Protein-2.

Introduction
Premature canities is a term for graying hair that occurs earlier than the usual age of onset, that is, before the 20 years old in Caucasians, 25 years old in Asians, and 30 years old in African–Americans [1,2]. The prevalence of premature canities in women is slightly higher (51.9%) than in men (48.1%) [3]. Premature canities occur because of the exhaustion of the ability to regenerate hair pigmentation and premature degenerative changes in melanocytes. Risk factors for premature canities that have been reported include oxidative stress, other comorbid diseases (pernicious anemia, hypo/hyperthyroidism, and cardiovascular disease), nutritional deficiency, and consumption of drugs (chloroquine, mephenesin, phenylthiourea, triparanol, fluorobutyrophenone, dixyrazine, and interferon alfa) [4]. Premature canities that occur at a relatively early age have a significant adverse effect on appearance, self-esteem, sociocultural acceptance, as well as the psychological and quality of life [4-7].

Micronutrient deficiency causes a wide spectrum of clinical manifestations in skin and hair, namely, loss of hair pigment [2,8,9]. Vitamin B12 and folic acid play an important role in the proliferation of hair follicle cells in the synthesis of DNA [10]. Krugluger et al. concluded that iron and vitamin B12 contribute to the physiology of growth and melanogenesis in hair [11]. The binding of copper, iron, zinc, and calcium ions also plays an important role in melanogenesis [12,13]. Supplementation is relatively affordable and readily available, but knowing what vitamins and minerals can help in treating premature canities is important. The management of premature canities remains limited and far from satisfactory [4,14]. Clinical research on premature canities also takes a long time, and the results are unsatisfactory. This study aims to analyze the relationship between serum micronutrient levels and premature canities.

Methods
Literature Search
The MEDLINE/PubMed electronic database, Cochrane library, ClinicalTrials.gov, EBSCO, Scopus, ProQuest, and Cambridge Core. Reference lists, conference proceedings, researchers in fields of eligible studies were searched. The following Mesh terms were used for searching: ("premature graying of hair" OR "premature canities") AND ("micronutrient" OR "trace elements" OR "vitamin B12" OR "ferritin" OR "iron" OR "calcium" OR "zinc" OR "copper"). A literature search was performed by three reviewers independently using PRISMA flow diagram 2009 [15]. Differences in opinion were resolved between all reviewers to reach a consensus.

Inclusion criteria include observational studies regarding the relationship between serum micronutrient levels with premature canities from 2012 until 2020, age between 20 and 30 years, and participants having at least five gray hairs, not suffering from canities caused by other conditions (genetic disorders of a premature aging syndrome, suffering from autoimmune comorbid diseases, hypomelanotic hair disorders, silver hair syndrome, Menkes syndrome, and metabolic syndrome), and not taking vitamins and mineral supplements or other drugs. Studies were excluded if they were written neither in Indonesian nor in English, were case reports, serial cases, letters, literature reviews, or systematic reviews.

Study Selection
Three reviewers conducted the study selection independently. Duplicate articles were removed. Using the predefined inclusion and exclusion criteria, title/abstract reviews and full-text reviews were assessed for eligibility. To reach a consensus, differences in opinion were resolved between all reviewers.

Data Extraction
Data were extracted independently by three reviewers using a modified The Cochrane Collaboration data collection form [16] Differences in opinion during data extraction were resolved between all reviewers and consensus was reached.

Assessment of Study Quality
Study quality assessment was performed independently by three reviewers using STROBE Statement and STROBE-Modified [17–19].

Data Synthesis
Meta-analysis difference in weighted mean was conducted using comprehensive meta-analysis: a computer program for meta-analysis, version 3.3. A descriptive synthesis was performed where data were not available to enable pooling.

Result
Initial database searches identified 109 nonduplicate records, of which 87 and 10 were excluded during the title/abstract and full-text review assessment. Twelve studies were included in this review, of which nine were included for meta-analysis. Figure 1 gives details of the study selection process.

Study Characteristics
Table 1 presents the characteristics of included studies. Most studies were conducted in India (n = 8), followed by Egypt (n = 2), Iran (n = 1), and Indonesia (n = 1) between 2012 until 2020. Twelve studies (n = 1791) were included in qualitative analysis of which nine (n = 1381) were included in meta-analysis. Serum levels of micronutrients vitamin B12, iron, ferritin, copper, calcium, and zinc were compared in the premature canities and control groups.

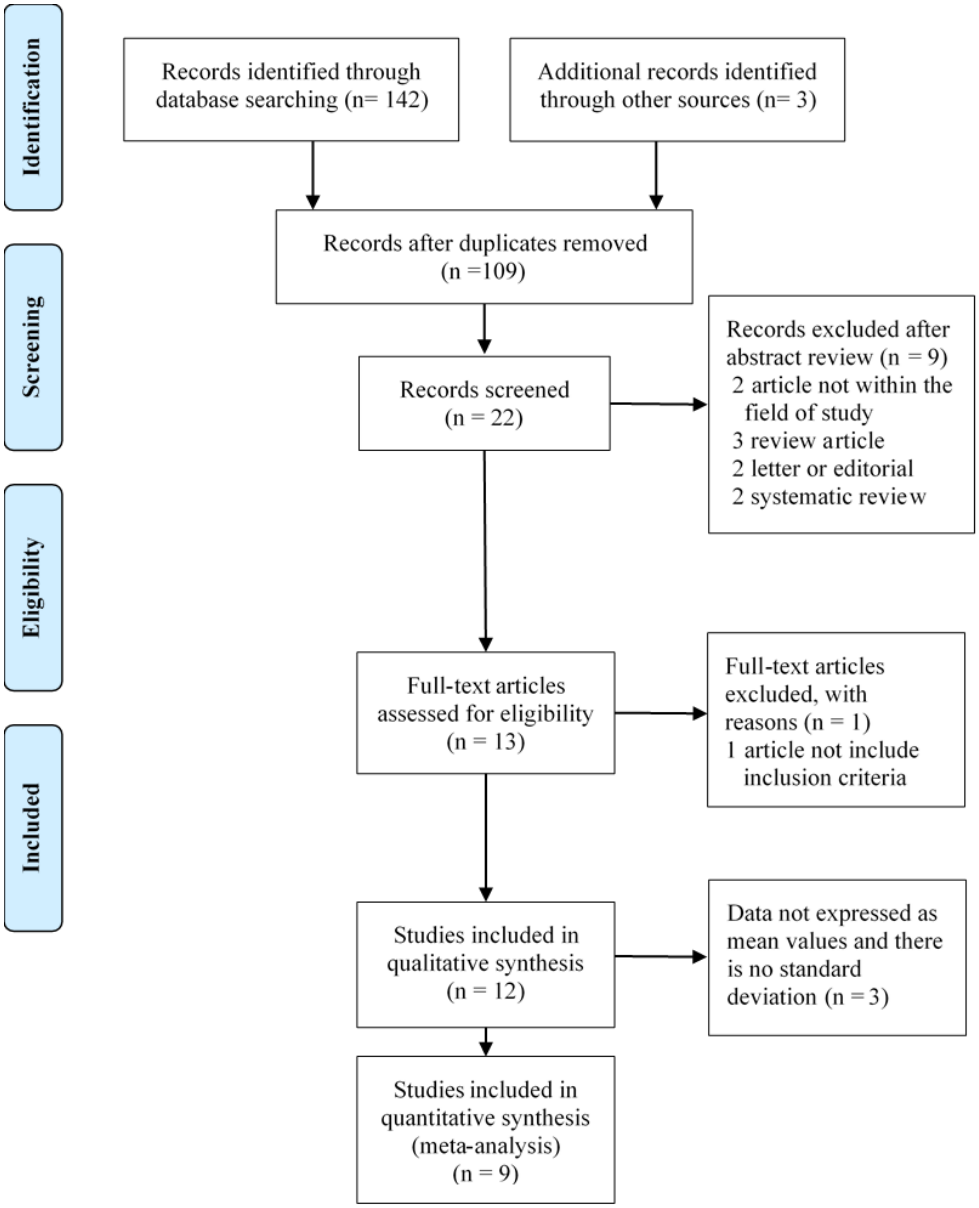


Figure 1: PRISMA flow diagram.

Quality of Study in Included Studies

The study quality assessment of the research articles included in this systematic review and meta-analysis comprised four cross-sectional studies and eight case–control studies. There were only four out of eight case–control study articles that stated the number of subjects with micronutrient deficiency; hence, the number of risk factors for micronutrient deficiency in premature canities could not be determined [22,25,26,28].

The overall study quality assessment was stated to have good quality comprising 10 research articles (83.3%) and fair quality comprising two research articles (16.7%). Sonthalia et al. had fair quality. Sonthalia et al. did not explain clearly the research design used, and certificates of ethical approval and patient informed consent were not listed in the research article. Additionally, Sonthalia et al. only had a case group with premature canities and did not have a control group as a comparison [23]. Anggraini et al. also had fair quality, because they did not explain the existence of informed consent and did not have a control group as a comparison. Both studies used a cross-sectional study [27]. Tables 2 and 3 show the assessments of the quality of the included studies.

Meta-analysis

Meta-analysis weighted mean difference of micronutrient levels between premature canities group compared with nonpremature canities found lower level in premature canities group than in nonpremature canities group (Figure 2). The overall effect of mean difference serum vitamin B12 level was −0.697 ± 0.105 (95% CI = −0.904 to −0.490), a negative value indicating that the serum vitamin B12 level in the premature canities group was significantly lower than that in controls ($p < 0.001$). The overall mean difference serum iron level was 0.146 ± 0.105 (95% CI = −0.058 to 0.350), a

Table 1: Characteristics of included studies.

No.	Study	Country	Total sample (n)	Case (n)	Controls (n)	Cases (Age, years mean ± SD or range)	Controls (Age, years mean ± SD or range)	Study design	Micronutrient levels
1.	Naieni et al, 2012 [20]	Iran	132	66	66	17.8 ± 2.0	18.3 ± 1.5	Cross-sectional study	Iron, copper, zinc
2.	Bhat RM et al, 2013 [2]	India	70	35	35	16.8	16.8	Epidemiological and investigative study	Vitamin B12, ferritin, iron, calcium
3.	Bhat YJ et al, 2016 [21]	India	100	50	50	9 to 13	9 to 13	Cross-sectional study	Ferritin, iron, calcium
4.	Chakrabarty et al, 2016 [1]	India	74	37	37	22.8 ± 1.7	22.8 ± 1.8	Case–control study	Vitamin B12, ferritin, copper, zinc, calcium,
5.	Daulatabad et al, 2017 [22]	India	104	52	52	14.5 ± 4.2	14.6 ± 2.9	Case control study	Vitamin B12, biotin, folic acid
6.	Sonthalia et al, 2017 [23]	India	71	71	0	5 to 19	-	Retrospective study	Vitamin B12, ferritin
7.	El-Sheikh et al, 2018 [24]	Mesir	90	60	30	5 to 30	14 to 30	Case–control study (design is not stated explicitly)	Iron, copper, calcium
8.	Sharma et al, 2018(a) [25]	India	120	60	60	<25	<25	Case–control study	Vitamin B12
9.	Sharma et al, 2018(b) [26]	India	240	120	120	15.71	15.91	Case–control study	Calcium, ferritin, vitamin B12
10.	Anggraini et al, 2019 [27]	Indonesia	100	100	0	20.09 ± 2.01	–	Cross-sectional study	Ferritin
11.	El-Husseiny et al, 2020 [28]	Mesir	600	300	300	22 ± 3	24 ± 2	Case–control cross-sectional study	Ferritin
12.	Brar et al, 2020 [9]	India	90	60	30	17.62	17.62	Case–control study (design is not stated explicitly)	Vitamin B12, ferritin

Table 2: STROBE-M *Quality Rating Sheet (Cross-sectional Study*) [19].

S Item	Scoring scale	Maximum possible Score	Naieni, 2012	Bhat YJ, 2016	Sonthalia, 2017	Anggraini, 2019
1.1	Study design mentioned. Yes = 1, No = 0	1	1	1	1	1
1.2	Background explained. Yes = 1, No = 0	1	1	1	1	1
1.3	Objective mentioned. Yes = 1, No = 0	1	1	1	1	1
1.4	Participant selection explained. Yes = 1, No = 0	1	1	1	1	1
1.5	Methods written. Yes = 1, No = 0	1	1	1	1	1
1.6	Results stated. Yes = 1, No = 0	1	1	1	1	1
1.7	Conclusions mentioned. Yes = 1, No = 0	1	1	1	1	1
2.1	Overview of known information. Yes = 1, No = 0	1	1	1	1	1
2.2	Recent pertinent references used. Yes = 1, No = 0	1	1	1	1	1
2.3	Gaps in current knowledge addressed. Yes = 1, No = 0	1	1	1	1	1
3.1	Populations specified. Yes = 1, No = 0	1	1	1	1	1
3.2	Exposures stated. Yes = 1, No = 0	1	1	1	1	1
3.3	Expected outcomes mentioned. Yes = 1, No = 0	1	1	1	1	1
3.4	Parameters to be estimated mentioned. Yes = 1, No = 0	1	1	1	1	1
4.1	Study design explicitly stated. Yes = 1, No = 0	1	1	1	0	1
4.2	Cohort population explained. Yes = 1, No = 0	1	0	0	0	0
4.3	Institutional Review Board / Ethics committee permission mentioned. Yes = 1, No = 0	1	1	1	0	1
4.4	Informed consent taken from participants. Yes=1, No = 0	1	1	1	0	0
5.1	Study setting mentioned. Yes = 1, No = 0	1	1	1	1	1
5.2	Study location written. Yes = 1, No = 0	1	1	1	1	1
5.3	Relevant dates mentioned (recruitment, exposure, follow-up, data collection). Yes=1-4, No = 0	4	1	1	1	1
6.1 Cross sectional	Eligibility criteria stated. Yes = 1, No = 0	1	1	1	1	1
6.2 Cross sectional	Sources and methods of selection of participants mentioned. Yes = 2, only source or method = 1, No = 0	2	2	1	1	1
7.1	Outcome variable/s is/are defined? Yes = 1, No = 0	1	1	1	1	1
7.2	Exposures defined? Yes = 1, No = 0	1	1	1	1	1
7.3	Predictors defined? Yes = 1, No = 0	1	1	1	1	1

7.4	Potential Confounders defined? Yes = 1, No = 0	1	1	1	0	0
7.6	Diagnostic Criteria defined? Yes = 1, No = 0	1	1	1	1	1
8.1	For each variable of interest sources of data given. Yes = 1, No = 0	1	1	1	1	1
8.2	For each variable of interest methods of assessment (measurement) mentioned. Yes = 1, No = 0	1	1	1	1	1
8.3	For each variable of interest comparability of assessment methods described (If there is more than one group). Yes = 1, No = 0	1	1	1	0	0
9.0	Efforts to address potential sources of bias are described. Yes = 1, No = 0	1	1	1	0	0
10.1	Known prevalence of variable from literature stated. Yes = 1, No = 0	1	1	1	1	1
10.2	Required significance mentioned. Yes = 1, No = 0	1	1	1	1	1
10.3	Required power mentioned. Yes = 1, No = 0	1	0	0	0	0
11.1	Explained how quantitative variables were handled in the analyzes. Yes = 1, No = 0	1	1	1	1	1
11.2	Described which groupings were chosen. Yes = 1, No = 0	1	1	1	1	1
11.3	Described why specific groupings were chosen. Yes = 1, No = 0	1	1	1	1	1
12.1	Statistical methods, including those used to control for confounding explained. Yes = 1, No = 0	1	1	1	1	1
12.2	Methods used to examine subgroups and interactions explained. Yes = 1, No = 0	1	1	1	1	1
12.3	Method to handle missing data addressed. Yes = 1, No = 0	1	0	0	0	0
12.4	For the cross-sectional study, described analytical methods taking account of sampling strategy. Yes = 1, No = 0	1	1	1	1	1
12.5	Sensitivity analyzes described. Yes = 1, No = 0	1	0	0	0	0
13.1	Numbers of eligible Individuals reported. Yes = 1, No = 0	1	1	1	1	1
13.2	Number of individuals Included in the Study reported. Yes = 1, No = 0	1	1	1	1	1
13.4	Number of individuals analyzed reported. Yes = 1, No = 0	1	1	1	1	1
13.5	Give reasons for nonparticipation at each stage. Yes = 1, No = 0	1	0	0	0	0
13.6	Recruitment and follow-up flow diagram presented. Yes = 1, No = 0	1	0	0	0	0
14.1	Characteristics (eg, Demographic, Clinical, Social) of study participants written. Yes = 1, No = 0	1	1	1	1	1
14.2	Information on potential confounders written. Yes = 1, No = 0	1	1	1	1	1
14.3	Number of participants with missing data for each variable of interest indicated. Yes = 1, No = 0	1	0	0	0	0
16.1	Given unadjusted estimates with precision (confidence interval). Yes = 1, No = 0	1	0	0	0	0
16.2	Given confounder-adjusted estimates (confidence interval). Yes = 1, No = 0	1	0	0	0	0
16.3	Described which confounders were adjusted for and why they were included. Yes = 1, No = 0	1	0	0	0	0
16.4	Reported category boundaries when continuous variables were categorized. Yes = 1, No = 0	1	1	1	1	1
16.5	Reported estimates of relative risk into absolute risk for a meaningful time period. Yes = 1, No = 0	1	0	0	0	0
17.0	Reported other analyzes done-eg, analyzes of subgroups and interactions, and sensitivity analyzes. Yes = 1, No = 0	1	1	1	1	1
18.0	Summarized key results with reference to study objectives. Yes = 1, No = 0	1	1	1	1	1
19.1	Discussed study limitations. Yes = 1, No = 0	1	1	1	1	1
19.2	Discussed direction and magnitude of potential bias. Yes = 1, No = 0	1	0	0	0	0
20.1	Presented a cautious overall interpretation considering objectives, Yes = 1, No = 0	1	1	1	1	1
20.2	Presented a cautious overall interpretation considering limitations, Yes = 1, No = 0	1	1	1	1	1
20.3	Presented a cautious overall interpretation considering the multiplicity of analyzes, Yes = 1, No = 0	1	1	1	1	1
20.4	Presented a cautious overall interpretation considering results from Similar Studies, Yes = 1, No = 0	1	1	1	1	1
20.5	Presented a cautious overall interpretation considering and other Relevant Evidence Yes = 1, No = 0	1	1	1	1	1
21.0	Discussed the generalizability (external validity) of the study results. Yes = 1, No = 0	1	1	1	1	1
22.1	Given the source of funding. Yes = 1, No = 0	1	0	0	1	0
22.2	Given the role of the funders for the present study. Yes = 1, No = 0	1	0	0	0	0
22.3	Stated author's and co-authors contributions. Yes = 1, No = 0	1	1	1	1	1
22.4	Given a statement on data available online. Yes = 1, No = 0	1	0	0	0	0
22.5	Given a statement on competing interests. Yes = 1, No = 0	1	1	0	1	0
	Total score = cross sectional (77)	**77**	**59**	**57**	**53**	**53**
	Percentage compliance (%)	**100**	**77**	**74**	**69**	**69**
	Quality grade = > 85% (excellent), 70 to <=85% (good), 50 to <70 (fair), <50 (poor)	**Excellent**	**Good**	**Good**	**Fair**	**Fair**

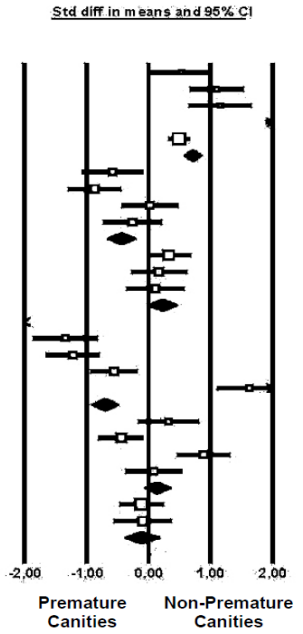
Table 3: STROBE-M *Quality Rating Sheet (Case–Control Study)* [19].

S Item	Scoring scale	Max score	Bhat RM, 2013	Chakra-barty, 2016	Daulata-bad, 2017	El-Sheikh, 2018	Sharma, 2018(a)	Sharma, 2018(b)	El-Husseiny, 2020	Brar, 2020
1.1	Study design mentioned. Yes = 1, No = 0	1	1	1	1	0	1	1	1	0
1.2	Background explained. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
1.3	Objective mentioned. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
1.4	Participant selection explained. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
1.5	Methods written. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
1.6	Results stated. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
1.7	Conclusions mentioned. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
2.1	Overview of known information. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
2.2	Recent pertinent references used. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
2.3	Gaps in current knowledge addressed. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
3.1	Populations specified. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
3.2	Exposures stated. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
3.3	Expected outcomes mentioned. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
3.4	Parameters to be estimated mentioned. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
4.1	Study design explicitly stated. Yes = 1, No = 0	1	1	1	1	0	1	1	1	0
4.2	Cohort population explained. Yes = 1, No = 0	1	0	0	0	0	0	0	1	0
4.3	Follow-up time period stated. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
4.4	Institutional Review Board/Ethics Committee permission mentioned. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
4.5	Informed consent taken from participants. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
5.1	Study setting mentioned. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
5.2	Study location written. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
5.3	Relevant dates mentioned (recruitment, exposure, follow-up, data collection). All four dates mentioned=4, few dates mentioned (1 to 3) = 1 to 3, No = 0	4	3	1	0	1	1	1	1	1
6.1 Case control	Eligibility criteria stated. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
6.2 Case Control	Sources and methods of case ascertainment and control selection written. Yes = 2, only source or method Yes = 1, No = 0	2	2	2	2	1	1	1	1	1
6.3 Case control	Rationale for the choice of cases and controls stated. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
6.4 Case control	For matched studies give matching criteria. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
6.5 Case control	For matched studies give number of controls per case. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
7.1	Outcome variable/s is/are defined? Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
7.2	Exposures defined? Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
7.3	Predictors defined? Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
7.4	Potential Confounders defined? Yes = 1, No = 0	1	1	0	1	1	1	1	1	0
7.5	Effect Modifiers defined? Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
7.6	Diagnostic Criteria defined? Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
8.1	For each variable of interest sources of data given. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
8.2	For each variable of interest methods of Assessment (measurement) mentioned. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
8.3	For each variable of interest Comparability of assessment methods described (If there is more than one group). Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
9.0	Efforts to address potential sources of bias described. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
10.1	Known prevalence of variable from literature stated. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1

10.2	Required significance mentioned. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
10.3	Required power mentioned. Yes = 1, No = 0	1	0	0	0	0	0	0	0	0
11.1	Explained how quantitative variables were handled in the analyzes. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
11.2	Described which groupings were chosen. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
11.3	Described why specific grouping were chosen. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
12.1	Statistical methods, including those used to control for confounding explained. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
12.2	Methods used to examine subgroups and interactions explained. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
12.3	Method to handle missing data addressed. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
12.4	For Case–control study, explained how matching of cases and controls was addressed. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
12.5	Sensitivity analyzes described. Yes = 1, No = 0	1	0	0	0	0	0	0	0	0
13.1	Numbers of eligible Individuals reported. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
13.2	Number of individuals Included in the Study reported. Yes = 1, No = 0,	1	1	1	1	1	1	1	1	1
13.3	Number of individuals completing follow-up reported. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
13.4	Number of individuals analyzed reported. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
13.5	Give Reasons for Nonparticipation at each stage. Yes = 1, No = 0	1	0	1	1	0	0	0	0	0
13.6	Recruitment and follow-up flow diagram presented. Yes = 1, No = 0	1	0	0	0	0	0	0	0	0
14.1	Characteristics (eg, Demographic, Clinical, Social) of study participants written. Yes = 1, No = 0	1	1	1	1	1	0	1	1	1
14.2	Information on exposures and potential confounders written. Yes = 1, No = 0	1	0	0	1	1	1	1	1	0
14.3	Number of participants with missing data for each variable of interest indicated. Yes = 1, No = 0	1	0	1	0	0	0	0	0	0
15.0	Case–control Study: Numbers in each exposure category, or summary measures of exposure reported. Yes = 1, No = 0	1	0	0	1	0	1	1	1	0
16.1	Given unadjusted estimates with precision (confidence interval). Yes = 1, No = 0, NA	1	0	0	0	0	0	0	0	0
16.2	Given confounder-adjusted estimates (confidence interval). Yes = 1, No = 0	1	0	0	0	0	0	0	0	0
16.3	Described which confounders were adjusted for and why they were Included. Yes = 1, No = 0	1	0	0	1	1	1	1	1	0
16.4	Reported category boundaries when continuous variables were categorized	1	1	1	1	1	1	1	1	1
16.5	Reported estimates of relative risk into absolute risk for a meaningful time period. Yes = 1, No = 0	1	0	0	0	0	0	0	0	0
17.0	Reported other analyzes done—eg, analyzes of subgroups and interactions, and sensitivity analyzes. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
18.0	Summarized key results with reference to study objectives. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
19.1	Discussed study limitations. Yes = 1, No = 0	1	0	0	1	0	0	1	1	1
19.2	Discussed direction and magnitude of potential bias. Yes = 1–2, No = 0	2	2	1	2	2	1	2	2	2
20.1	Presented a cautious overall interpretation considering objectives, Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
20.2	Presented a cautious overall interpretation considering limitations, Yes = 1, No = 0	1	0	1	1	0	0	1	1	1
20.3	Presented a cautious overall interpretation considering multiplicity of Analyzes, Yes = 1, No = 0	1	1	1	1	1	1	1	1	1

20.4	Presented a cautious overall interpretation considering results from Similar Studies, Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
20.5	Presented a cautious overall interpretation considering and other Relevant Evidence Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
21.0	Discussed the generalizability (external validity) of the study results. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
22.1	Given the source of funding. Yes = 1, No = 0	1	0	1	1	1	0	1	0	0
22.2	Given the role of the funders for the present study. Yes = 1, No = 0	1	0	0	0	0	0	0	0	0
22.3	Stated author's and co-authors contributions. Yes = 1, No = 0	1	1	1	1	1	1	1	1	1
22.4	Given a statement on data available online. Yes = 1, No = 0	1	0	0	0	0	0	0	0	0
22.5	Given a statement on competing interests. Yes = 1, No = 0	1	0	1	1	1	0	1	0	0
	Total score	**83**	**64**	**65**	**69**	**63**	**62**	**68**	**67**	**60**
	Percentage compliance	**100**	**77**	**78**	**83**	**76**	**75**	**82**	**81**	**72**
	Quality grade => 85% (excellent), 70 to <=85% (good), 50 to <70 (fair), <50 (poor)	**Excellent**	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**	**Good**

Group by Subgroup wit	Study name	Subgroup within study	Statistics for each study						
			Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Feritin	Bhat RM, 2013	Feritin	0,534	0,243	0,059	0,058	1,011	2,197	0,028
Feritin	Bhat YJ, 2016	Feritin	1,098	0,215	0,046	0,677	1,518	5,118	0,000
Feritin	Chakrabarty S, 2016	Feritin	1,155	0,251	0,063	0,663	1,648	4,601	0,000
Feritin	Brar BK, 2020	Feritin	2,478	0,290	0,084	1,909	3,046	8,544	0,000
Feritin	El-Husseiny, 2020	Feritin	0,494	0,083	0,007	0,331	0,656	5,955	0,000
			0,720	0,069	0,005	0,585	0,854	10,476	0,000
Kalsium	Bhat RM, 2013	Kalsium	-0,577	0,244	0,060	-1,055	-0,099	-2,365	0,018
Kalsium	Bhat YJ, 2016	Kalsium	-0,871	0,209	0,044	-1,281	-0,461	-4,163	0,000
Kalsium	El-Sheikh MA, 2018	Kalsium	0,023	0,224	0,050	-0,416	0,461	0,101	0,919
Kalsium	Chakrabarty S, 2016	Kalsium	-0,255	0,233	0,054	-0,712	0,203	-1,090	0,276
			-0,433	0,113	0,013	-0,655	-0,212	-3,828	0,000
Tembaga	Naieni FF, 2012	Tembaga	0,330	0,175	0,031	-0,014	0,673	1,882	0,060
Tembaga	El-Sheikh MA, 2018	Tembaga	0,175	0,224	0,050	-0,264	0,614	0,780	0,435
Tembaga	Chakrabarty S, 2016	Tembaga	0,113	0,238	0,054	-0,349	0,589	0,487	0,626
			0,230	0,119	0,014	-0,003	0,463	1,936	0,053
Vitamin B12	Bhat RM, 2013	Vitamin B12	-2,914	0,343	0,118	-3,587	-2,242	-8,491	0,000
Vitamin B12	Chakrabarty S, 2016	Vitamin B12	-1,344	0,257	0,066	-1,849	-0,839	-5,221	0,000
Vitamin B12	Daulatabad D, 2017	Vitamin B12	-1,217	0,213	0,046	-1,635	-0,798	-5,700	0,000
Vitamin B12	Sharma N, 2018	Vitamin B12	-0,556	0,186	0,035	-0,920	-0,191	-2,986	0,003
Vitamin B12	Brar BK, 2020	Vitamin B12	1,628	0,254	0,065	1,129	2,126	6,399	0,000
			-0,697	0,105	0,011	-0,904	-0,490	-6,606	0,000
Zat besi	Bhat RM, 2013	Zat besi	0,326	0,241	0,058	-0,145	0,798	1,357	0,175
Zat besi	Naieni FF, 2012	Zat besi	-0,441	0,178	0,031	-0,787	-0,096	-2,505	0,012
Zat besi	Bhat YJ, 2016	Zat besi	0,869	0,210	0,044	0,479	1,300	4,242	0,000
Zat besi	El-Sheikh MA, 2018	Zat besi	0,091	0,224	0,050	-0,348	0,529	0,405	0,685
			0,146	0,104	0,011	-0,058	0,350	1,402	0,161
Zink	Naieni FF, 2012	Zink	-0,111	0,174	0,030	-0,452	0,231	-0,636	0,524
Zink	Chakrabarty S, 2016	Zink	-0,091	0,238	0,054	-0,547	0,365	-0,382	0,695
			-0,104	0,138	0,019	-0,377	0,170	-0,744	0,457



positive value indicated that the serum iron level of the premature canities group was lower than in controls, but the difference was not significant (p = 0.161). The overall mean difference serum ferritin level was 0.720 ± 0.071 (95% CI = 0.585–0.854), a positive value indicating that the serum ferritin level in the premature canities group was significantly lower than that in controls (p < 0.001).

The overall mean difference serum copper level was 0.230 ± 0.118 (95% CI = −0.003 to 0.463), the positive value indicated that the serum copper level of the premature canities group was lower than controls, but the difference was not significant (p = 0.053). The overall mean difference serum calcium level was −0.443 ± 0.114 (95% CI = −0.655 to −0.212), a negative value indicated that the serum copper level in the premature canities group was significantly lower than that in controls (p < 0.001). The overall mean difference serum zinc level was −0.104 ± 0.138 (95% CI = −0.377 to 0.170), a negative value indicating that the serum zinc level in the premature canities group was lower than that in controls, but the difference was not significant (p = 0.457). (Figure 2). The insignificant result might be due to the limited number of studies.

Discussion

This is a systematic review and meta-analysis to analyze lower serum micronutrient levels in premature canities compared to nonpremature canities. The overall study quality showed good results (83.3%). The association between premature canities and micronutrient deficiencies has been previously reported [9,20] The role of micronutrient deficiency has long been suggested to be involved in the etiopathogenesis of premature canities [2,8,30].

Bhat et al. have recommended screening micronutrient levels in patients with premature canities and subsequent supplementation therapy [2].

Serum vitamin B12 levels in the premature canities were significantly lower than controls. These data are in accordance with the literature stating that vitamins play a role in the pathogenesis of premature canities. Krugluger et al. concluded that iron and vitamin B12 contribute to the physiology of growth and melanogenesis in hair. Hair follicle cells are rapidly dividing cells, and their proliferation is dependent on DNA synthesis and an adequate supply of vitamin B12 and folic acid. Vitamin B12 plays a role in stabilizing the onset of the anagen phase of hair follicles [10,11] In current clinical practice, vitamin B12 is not routinely administered to patients with premature canities. These findings explain the role of vitamin B12 deficiency in the etiopathogenesis of premature canities and thus its possible role may be considered as nutritional supplementation in the treatment of premature canities. Pernicious anemia is also known to be associated with premature canities. It is not known whether the association between pernicious anemia and premature canities is due to an autoimmune etiology or through vitamin B12 [4,22].

Serum iron levels were lower in premature canities than in controls but not significantly different. The literature states that iron has a role in the etiopathogenesis of premature canities. Iron deficiency has been reported to cause scalp hair pigmentation disorders. Iron has also been reported to affect melanogenesis [12,31] Tyrosinase enzymes are required for the early stages of melanogenesis, where TRP-1 and TRP-2 require iron and zinc ions for stabilization and increase tyrosinase activity.12,13 Two tyrosinase-related proteins are as follows: (1) TRP-1 is required for tyrosinase activation and/or stabilization and converting 3,4-dihydroxyphenylalanine (DOPA)-quinone to eumelanin, whereas (2) TRP-2 converts DOPA-chrome to carboxylated derivative dehydroxyindole-2-carboxylic acid (DHICA). The role of iron ions has also been shown to modulate tyrosinase activity. Zinc and iron ions play a role in melanogenesis, namely, in the rearrangement of DOPA-chrome to 5,6-dihydroxyindoles and oxidative polymerization of 5,6-dihydroxyindoles to the melanin pigment. One of the advanced stages of melanin biosynthesis is the tautomerization reaction by DOPA-chrome tautomerase, which isomerization of DOPA-chrome to DHICA. This enzyme is a metalloenzyme with iron ions in its active site [12,13,20].

Serum ferritin levels were lower significantly in premature canities compared with controls. The results of this analysis are in accordance with the literature that iron also functions in the composition of enzymes in the process of hair pigmentation. Iron in the body is divided into three parts, which comprises (1) functional iron consisting of hemoglobin, myoglobin, and various types of enzymes, (2) reserve iron consisting of ferritin and hemosiderin, and (3) transport iron, namely, transferrin. Iron acts as a catalyst in oxidation and reduction reactions and controls DNA synthesis through the enzyme ribonuclease in cell division. Decreased bioavailability of iron is thought to interfere with the proliferation of follicular matrix cells [32,33] Therefore, ferritin, which is a reserve iron in the body, also plays an important role in the melanogenesis process of hair follicle cells [31].

Serum copper levels are lower in premature canities than in controls but not significantly different. The literature states that the tyrosinase enzyme is required for the early stages of melanogenesis. One of the most important enzymes in this reaction is the enzyme tyrosinase. Glycoproteins, located in the melanosomal membrane, consist of three domains: an inner melanosomal domain containing the catalytic region, a short transmembrane domain, and a cytoplasmic domain. Tyrosine is converted to DOPA by the enzyme tyrosinase. Copper ions bind to the interior of tyrosinase and are required for tyrosinase activity that converts L-DOPA to DOPA-quinone. Furthermore, two tyrosinase-associated proteins, TRP-1 and TRP-2, reach the melanosome membrane. TRP-1 is required for the activation and/or stabilization of tyrosinase, whereas TRP-2, which is required for the conversion of DOPA-chrome to the carboxylation derivative of DHICA, together with tyrosinase and TRP-1 [12,34].

Serum calcium levels were lower significantly in premature canities compared to controls. The results of this analysis are in accordance with the literature that premature canities have been associated with decreased bone mineral density. Calcium has also been involved in several stages of melanogenesis. Calcium ion is an important cofactor for protein kinase-C (PKC)α, PKCβ, and PKCγ, where PKCβ plays an important role in melanogenesis through phosphorylation and tyrosinase activation [12,35]. Rosen et al. hypothesized that premature canities are associated with osteopenia, suggesting a possible role for deficiency of vitamin D3 and calcium in the pathogenesis of premature canities [36].

Serum zinc levels are lower in premature canities than in controls but not significantly different. Besides vitamin B12, iron, copper, and calcium, zinc plays an important role in stabilizing and increasing tyrosinase activity in several stages of the melanogenesis process; moreover, TRP-2 requires iron and zinc ions for stabilization and increases tyrosinase activity. The enzymatic activity of TRP-2 requires metal ions such as zinc [12,13].

Our study had some limitations in the form of the number of research subjects regarding premature canities. Some studies were excluded from the meta-analysis because those were not able to compare with the control group or incompleted results. Hence, it was not possible to convert the yield to the expected micronutrient level yield. Additionally, not all research articles mentioned the number of subjects experiencing micronutrient deficiency; hence, the number of risk factors for micronutrient deficiency in patients with premature canities cannot be determined.

Conclusion

The meta-analysis showed that serum levels of vitamin B12, ferritin, and calcium were significantly lower in premature canities. Although the lower serum iron, copper, and zinc levels were

not significantly different in premature canities compared with nonpremature canities. Qualitative analysis found that deficiency of vitamin B12, iron, ferritin, zinc, copper, and calcium can affect the physiology of hair melanogenesis that causing premature canities. To assess the role of micronutrients in premature canities as well as the link between these variables, further study with a larger dataset is required.

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