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Original article

Salivary ferritin and iron as a marker and new discriminating indices between iron deficiency anemia and thalassemia: a meta-analysis

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Abstract: *Purpose* — We performed a meta-analysis of all eligible case-control studies published to date, to assess the association of salivary ferritin and iron with hematological disorders.
Methods — We conducted Medline/PubMed and Scopus searches for papers published from January 1, 1980, through January 1, 2013. Data were merged the summary mean difference were estimated using either a random-effects model or a fixed-effects model.
Results — There were 3 studies including 196 cases and 70 controls. There was a statistically significant difference in the salivary ferritin and iron between hematological diseases and control groups, and the summary estimate of mean difference was 1.37 (95%CI: 0.01–2.74) and 2.23 (0.92–3.54) in salivary ferritin and iron, respectively. The stratification showed the same statistically significant differences in the salivary ferritin between Iron deficiency anemia (IDA) with thalassemia intermedia (TI), and IDA with thalassemia major (TM) groups. Besides, the stratification showed that there were statistically significant differences in the salivary iron between IDA with TM groups, while no statistically significant difference was observed between IDA with TI.
Conclusion — Saliva ferritin iron and ferritin increase significantly in patients with thalassemia compared to IDA. Salivary ferritin and iron can be used as a diagnostic marker and new discriminating indices routinely to differentiate IDA from thalassemia, especially thalassemia trait; furthermore, their easy applicability, safe, relatively simple, and noninvasive procedure may be an important advantage compared to blood sample collection. Consequently, it could be promising to develop a simplified testing and differentiating procedure, which could reach many clinical decision-makers as a diagnostic potential.

Keywords: saliva, ferritin, iron, iron deficiency anemia, thalassemia intermedia, thalassemia major, discriminating indices

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Introduction

Anemia is defined as the presence of small or a decrease in a number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood. Thalassemia is a widespread hematologic abnormality that affects about 5% of the world population, and it is most prevalent in the Mediterranean countries [1]. Iron deficiency anemia (IDA) and thalassemia are groups of blood disorders with microcytic hypochromic manifestations [2, 3]. Laboratory diagnosis of thalassemia is a very serious concern due to increasing in need of prenatal diagnosis of hemoglobin chain disorders [4]. Iron is an essential element for all living organisms and it is the most important function is oxygen transport in hemoglobin. Iron deficiency anemia is usually caused by a broad spectrum of reasons such as malnutrition and is largely detected based on hematological laboratory findings [5]. Many discriminating indices in the distinction between IDA and thalassemia trait (TT) have been introduced using RBC indices so far [6, 7], which we have been reported the accuracy and clinical application of those indices, previously [8, 9]. Though these indices reported that were widely used in clinical decision-making and has several advantages, but the reliability of such indices is questionable and controversial [10].

Analyses of various body fluids are pushing the field of biomedical research forward due to its reliable ability to identify biochemical targets [11]. Saliva is one of the most important diagnostic body fluid, which has significant biochemical and logistical advantages compared with blood [12]. The collection of saliva is noninvasive, safe, and relatively simple, and may be done frequently without discomfort to the patient [13]. The potential advantage of this diagnostic body fluid could extend to clinical decision-making and health care [14]. However, the salivary iron and ferritin may consider as the potential and new discriminating indices with the advantage of an easy and noninvasive approach [15, 16].

It is important to perform a quantitative synthesis of the available evidence using more rigorous methods on the amounts of evidence have been accumulated so far. Therefore, we performed a meta-analysis of all eligible case-control studies published to date, to assess the association of salivary ferritin and iron with hematological disorders especially IDA and TT.

Table 1. Description of included studies (salivary ferritin)

Author, year	*Country*	*Sample Size*		*Salivary ferritin, ng/dl, M±SD*		*Type of hematologic disorder*	*Control group*
		Case	Control	Case	Control		
Canatan and Akdeniz, 2012 [25]	Turkey	30	35	18.6 ± 8.53	42.5 ± 42.25	IDA	Healthy
Canatan and Akdeniz, 2012 [25]	Turkey	71	35	2529.6 ± 1081.3	42.5 ± 42.25	TM	Healthy
Canatan and Akdeniz, 2012 [25]	Turkey	10	35	1166.0 ± 51.3	42.5 ± 42.25	TI	Healthy
Canatan and Akdeniz, 2012 [25]	Turkey	15	35	112.2 ± 145.9	42.5 ± 42.25	TT	Healthy
Jagannathan et al., 2012 [26]	India	30	30	15.32 ± 4.65	9.38±3.01	IDA	NM

M±SD is mean with standard deviation. IDA, iron deficiency anemia; TM, thalassemia major; TI, thalassemia intermedia; TT, thalassemia trait.

Table 2. Description of included studies (salivary iron)

Author, year	*Country*	*Sample Size*		*Salivary iron, mg/dl, M±SD*		*Type of hematologic disorder*	*Control group*
		Case	Control	Case	Control		
Canatan and Akdeniz, 2012 [25]	Turkey	30	35	24.6 ± 10.0	74.2 ± 40.7	IDA	Healthy
Canatan and Akdeniz, 2012 [25]	Turkey	71	35	253.6 ± 91.0	74.2 ± 40.7	TM	Healthy
Canatan and Akdeniz, 2012 [25]	Turkey	10	35	150.1 ± 61.1	74.2 ± 40.7	TI	Healthy
Canatan and Akdeniz, 2012 [25]	Turkey	15	35	101.1 ± 41.2	74.2 ± 40.7	TT	Healthy
Mishra et al., 1992 [27]	India	27	10	134.1 ± 39.3	103.9 ± 9.6	IDA	Non-anemic
Mishra et al., 1992 [27]	India	8	10	185.3 ± 47.6	103.9 ± 9.6	TM	Non-anemic
Mishra et al., 1992 [27]	India	5	10	228.8 ± 51.9	103.9 ± 9.6	AA	Non-anemic

M±SD is mean with standard deviation. IDA, iron deficiency anemia; TM, thalassemia major; TI, thalassemia intermedia; TT, thalassemia trait; AA, aplastic anemia.

Patients and Methods

Literature source and searching methods

We performed Medline/PubMed and Scopus searches using Mesh terms including ((((("Saliva"[Mesh]) AND "Ferritins"[Mesh]) OR "Iron"[Mesh]) AND "Anemia, Iron-Deficiency"[Mesh]) OR "Thalassemia"[Mesh] for papers published (from January 1, 1980 through May 1, 2013). Further publications were also recognized by retrieving the bibliographies of the retrieved papers through reference check method.

Study design and selection

Case-control studies were included in the evaluation since this study design allows a comparison to be made between the affected individuals and healthy or disease-free ones, which is essential for the meta-analysis model. Studies that measured salivary ferritin and iron and included patients with any hematological condition such as IDA, TT, thalassemia major (TM), thalassemia trait (TT), thalassemia intermedia (TI) and aplastic anemia (AA), were included in the evaluation. The papers that published in English, offer the size of the sample, arithmetic means and standard deviations (SD) or the information that can help infer the results were included. Studies that were not representative or not case-control were excluded. The studies that contain not adequate data for analysis were excluded after contacting the corresponding author twice.

Data extraction

Two researchers reviewed the selected publication individually and reached an agreement on all of the eligibility items, including author, journal and year of publication, the location of study, selection and characteristics of cases and controls, control source, and demographics information.

Meta-analysis

We estimated the summary arithmetic means and standard deviations (SMD), if the study provided stratum information, the data coming from similar stratum were added up to make a full use. Both Der Simonian and Laird's random-effects method and Mantel–Haenszel's fixed-effects method were used [17]. In the meta-analysis, to evaluate the between-study heterogeneity both chi-square-based Q-statistic and I-squared (I^2) tests were performed. Furthermore, according to Venice criteria, for the I^2 test included: <25% represents no heterogeneity, =25–50% represents moderate heterogeneity, =50-75% represents large heterogeneity, and >75% represents extreme heterogeneity. So the heterogeneity was considered significant, if the P<0.10 and I^2>25, a random-effect model was suitable, otherwise if the P≥0.10 and I^2≤25, a fixed-effect model was then used to estimate summary odds ratios (Ors) and 95% confidence intervals (Cis). Publication bias was assessed by a funnel plot based on the Egger's regression test, and t-test was implemented to determine the significance of the asymmetry [18-24].

Statistical analysis

All of the statistical analyses were performed with STATA 11.0 software package (Stata Corporation, College Station, Texas, USA). All the tests were two-side, a P value of less than 0.05 for any test or model was considered to be statistically significant.

Results

Meta-analysis

There were 3 studies including 196 cases and 70 controls (*Figure* 1) [25-27]. The characterization of all selected publications was summarized in *Tables* 1 and 2. Two combined analysis include seven individual case-control studies, were included [25, 27].

Test of heterogeneity

The heterogeneity of studies on salivary ferritin and iron was analyzed for the 3 selected studies, separately. The results show that all meta-analysis on salivary ferritin and Iron had heterogeneity with a P-value less than 0.05 (*Figure* 2).

Table 3. Summary results of meta-analysis on salivary ferritin and iron in iron deficiency anemia and thalassemia

Population	*Case/Control (type of model)*	*Heterogeneity test* Q	P	*Hypothesis test* Z	P	I^2	*Egger test* t	P	*Begg test* Z	P	*Summary estimates of mean difference (95% CI)*
Salivary ferritin	156/170 (Random)	101.23	<0.0001	1.97	0.049	96%	0.94	0.418	0.49	0.625	1.37 (0.01–2.74)
Salivary iron	166/170 (Random)	113.43	<0.0001	3.34	0.001	94.7%	1.61	0.168	0.75	0.453	2.23 (0.92–3.54)
Salivary ferritin (stratification by hematologic disorder type)											
IDA w ith TI	60/20 (Fixed)	0.0	0.975	7.59	<0.0001	0.0%	-	-	0.00	1.00	2.49 (1.85–3.13)
IDA w ith TM	60/142 (Fixed)	0.0	0.993	13.33	<0.0001	0.0%	-	-	0.00	1.00	2.76 (2.36–3.17)
Salivary iron (stratification by hematologic disorder type)											
IDA w ith TI	57/20 (Random)	28.60	<0.001	1.17	0.241	96.4%	-	-	-1.00	0.317	2.17 (-1.45–5.80)
IDA w ith TM	57/81 (Random)	12.27	<0.001	2.42	0.015	91.8%	-	-	1.00	0.317	2.13 (0.41–3.85)

IDA, iron deficiency anemia; TM, thalassemia major; TI, thalassemia intermedia.

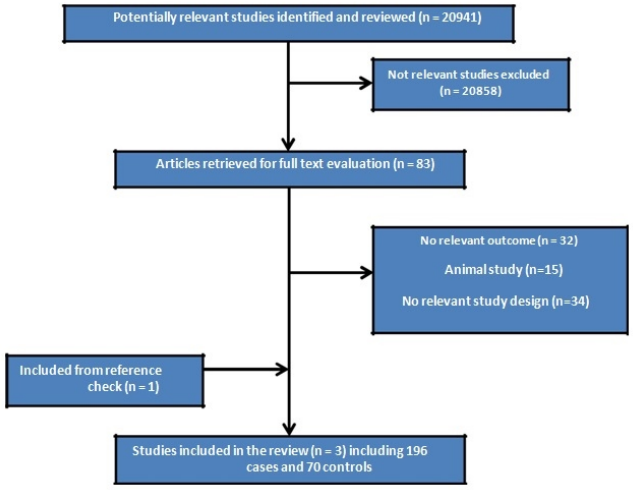

Figure 1. The flow diagram of study selection.

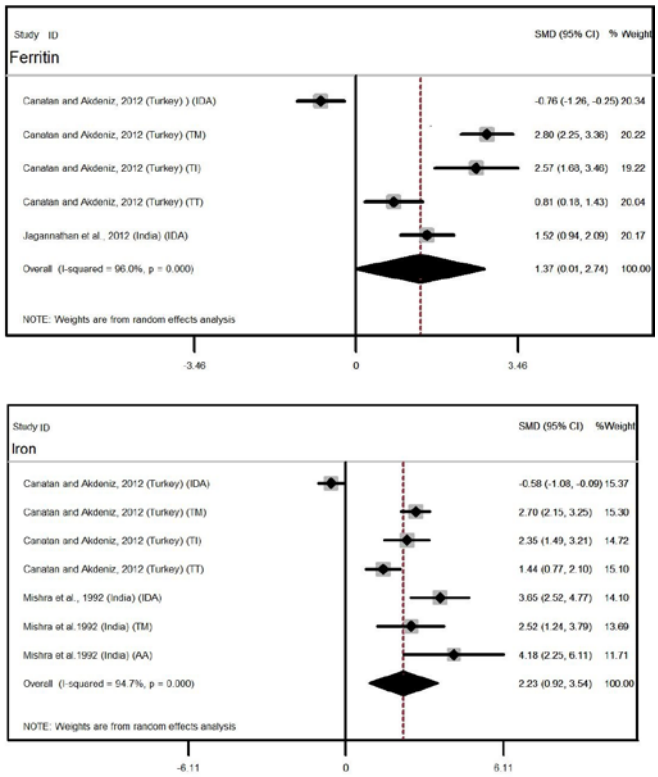

Figure 2. Meta-analysis is conducted on ferritin and iron among total hematologic disorders population. Each estimate of mean difference on cadmium is designated by a solid square, and the 95% confidence interval (95% CI) of each subgroup is shown by trans vers e line. The solid rhombus at the bottom is the pooled estimate of mean difference by fixed and random effects model. IDA, iron deficiency anemia; TM, thalassemia major.

Quantitative data synthesis

Therefore, we estimated the summary mean difference for them using a random-effects model (*Table* 3). There was a statistically significant difference in the salivary ferritin and iron between hematological diseases and control groups, and the summary estimate of mean difference was 1.37 (95%CI: 0.01–2.74) and 2.23 (0.92–3.54) in salivary ferritin and iron, respectively (*Figure* 2). We stratified the studies by hematological disorder type (*Figures* 3 and 4). The stratification showed that there were statistically significant differences in the salivary ferritin between IDA with TI, and IDA with TM groups and the summary estimates of mean difference were 2.49 (1.85–3.13) and 2.76 (2.19–3.17), respectively (*Table* 3 and *Figure* 3). Besides, the stratification showed that there were statistically significant differences in the salivary iron between IDA with TM groups, while no statistically significant difference was observed between IDA with TI and the summary estimates of mean difference were 2.17 (-1.45–5.80) and 2.13 (0.41–3.85), respectively (*Table* 3 and *Figure* 4).

Sensitivity analysis

We conducted the sensitivity analysis and found that subgroup analysis based on hematological disorder types (IDA versus thalassemia) and salivary content (ferritin venous iron) did make a noticeable difference for the above analyses (*Table* 3).

Assessing publication bias

Publication bias was evaluated by Egger's test and Begg's test (*Table* 3). Both tests suggest that publication bias might not have a significant influence on the summary estimate of salivary ferritin and iron among hematological disorders, and between different salivary content. Maybe, there was publication bias in a meta-analysis for the total population, because there was some uncertainty with the P-value being less than 0.05 in either Egger's or Begg's tests.

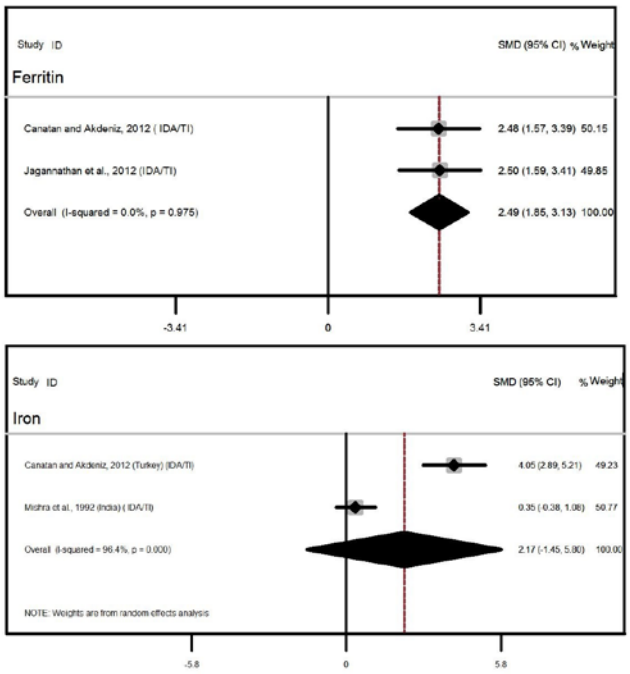

Figure 3. Meta-analysis is conducted on ferritin and iron among total hematologic disorders population. Each estimate of mean difference on cadmium is designated by a solid square, and the 95% confidence interval (95% CI) of each subgroup is shown by trans vers e line. The solid rhombus at the bottom is the pooled estimate of mean difference by fixed and random effects model. IDA, iron deficiency anemia; TI, thalassemia intermedia.

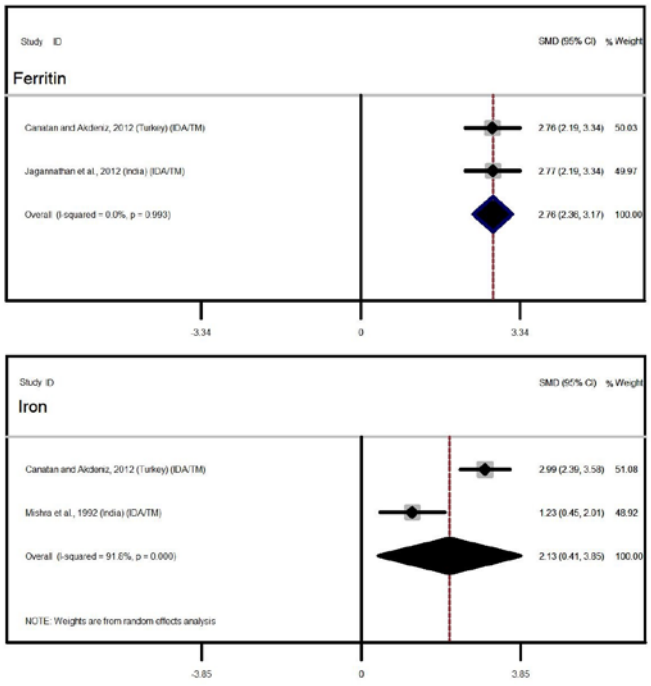

Figure 4. Meta-analysis is conducted on ferritin and iron among total hematologic disorders population. Each estimate of mean difference on cadmium is designated by a solid square, and the 95% confidence interval (95% CI) of each subgroup is shown by trans vers e line. The solid rhombus at the bottom is the pooled estimate of mean difference by fixed and random effects model. IDA, iron deficiency anemia; TM, thalassemia major.

Discussion

Meta-analyses and systematic reviews have the potential to provide the highest levels of evidence as a well-designed tool [28]. In the present meta-analysis, we observed the possibility of using the salivary ferritin and iron as a discriminating index between IDA and thalassemia with a certain focus on TT by performing a quantitative analysis of the published studies, in which the pooled results suggested a beneficial role.

A bunch of discrimination indices has been introduced to differentiate IDA from TT, formerly [29-35]. Many types of researche have been used these discriminating indices and reported various controversial results [36-41]. Given the availability of hemoglobin electrophoresis, these indices are not very imperative currently. The most important question is that, how many of physicians remember such formulas and use them in their daily practice in crowded patient settings? Besides the reliability of these indices has been a matter of controversy [10, 42, 43]. Furthermore, how many of medical specialists would be fearless enough to not study hemoglobin electrophoresis in certain or critical medical cases such as a pregnant woman with mild hypochromic anemia unresponsive to iron therapy? Moreover, these indices need blood sample that is the invasive method and blood itself is a far more complex medium. Hence, the collection of saliva, a clear liquid, is a safe, relatively simple, and noninvasive method, and requires no highly trained medical staff to collect. Our meta-analysis showed that salivary ferritin and iron could play a crucial role as discriminating indices between IDA and TT. The calculation of these discriminating indices is based on the RBCs characteristics, so variation in such indices may affect the clinical decision-making based on these tools. The RBC indices such as mean corpuscular volume (MCV) are differing in an age-dependency manner [8, 9, 44]. While, in the case of using salivary ferritin and iron as discriminating indices no significant differences were observed between the subgroups depending on sex and on age, formerly [45].

Conclusion

Saliva ferritin iron and ferritin increase significantly in patients with thalassemia compared to IDA. Salivary ferritin and iron can be used as a diagnostic marker and new discriminating indices routinely to differentiate IDA from thalassemia, especially TT; furthermore, their easy applicability, safe, relatively simple, and noninvasive procedure may be an important advantage compared to blood sample collection. Consequently, it could be promising to develop a simplified testing and differentiating procedure, which could reach many clinical decision-makers as a diagnostic potential.

Conflict of interest: none declared.

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