Comparative Diagnostic Utility of Mean Corpuscular Volume and Serum Ferritin in Iron Deficiency Anemia: A Cross-Sectional Study in a Saudi Population

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ABSTRACT

The study seeks to evaluate comparative diagnostic utility of iron deficiency biomarkers, MCV and serum ferritin in relation to Saudi context and the impact of demographic factors on the diagnostic accuracy of these parameters. This study employs a cross-sectional study design using clinical patient data from a gastroenterology center in Riyad Saudi Arabia. 210 participants were selected using a strict inclusion criterion, patients with confirmed diagnosis of IDA at hemoglobin (Hb) levels below 12 g/dL and serum ferritin concentrations under 100 ng/mL and patients with any confounding chronic or inflammatory condition were excluded. SPSS was used for data analysis using descriptive statistics and inferential statistics.The study was conducted at gastroenterology center in Riyad Saudi Arabia, with data collected from July 2022 and July 2023. Findings revealed an inverse correlation between MCV and ferritin ($r = -0.337$, $p < 0.001$) along with MCV showing an inverse correlation against Hb ($r = -0.487$). Females were found to have decreased ferritin levels ($r = -0.460$, $p < 0.001$), however, ferritin levels were seen significantly increased with age ($r = 0.182$, $p< 0.008$). Additionally, 35.4% of the sample population reported using PPI which potentially increases IDA risk. The study demonstrates MCV and ferritin to be critical but not definitive biomarkers for IDA in Saudi Arabian context, which demands contextual interpretation particularly due to thalassemia and PPI confounders. Findings recommend using both biomarkers simultaneously for improved diagnosis and combining them with additional progressive tests and demographic-specific thresholds.

Keywords: ***Anemia, Iron-deficiency, Saudi Arabia, Ferritins, Biomarkers, Thalassemia***

INTRODUCTION

Iron Deficiency Anemia (IDA) is one of the common nutritional deficiencies globally, affecting approximately 1.2 billion people worldwide with a higher burden of prevalence in developing nations[1]. Due to insufficient iron for the production of red blood cells, there is a reduction in the concentrations of hemoglobin or erythrocyte volume, this iron deficiency results in IDA which is often exacerbated by chronic blood loss, limited iron intake or absorption, chronic inflammation or increased iron demand in the body[2]. 25-35% of the population of Saudi Arabia is affected by IDA against 5-8% impact in Western countries, which is reflective of an emerging and critical public health challenge[3]. Given the higher prevalence of IDA, its diagnosis poses several challenges such as difficulties in differentiating between true iron deficiency from other microcytic anemias like thalassemia or anemia of chronic diseases[4,5].

IDA has wider impacts not just clinically but economically as well, for instance, 50% of all anemia cases are leaned towards excessive burden on women of reproductive age or children under five[6]. Meanwhile in Saudi Arabia, a national epidemiological survey revealed a 32.4% of women aged 15 to 49 had IDA in 2020, which was characterized by elements such as multiparity, iron deficient dietary habits and restricted use of supplementation programs[3]. IDA is not only characterized by fatigue, it also involves impairment of cognitive functions[7], lowering work productivity[8] while exacerbating maternal and child mortality and morbidity[9]. Iron deficiency is responsible to impair brain functioning by lowering oxygen to brain regions in result of anemia-induces hypoxia as well as disrupting iron-dependent enzymatic processes which are crucial for neurotransmitter synthesis. This chronic iron deficiency exacerbates neurodegenerative changes including white matter disease and cortical atrophy, ultimately playing a role in cognitive decline[7,10]. Whereas the economic toll is equally concerning that despite growing socio-demographic index in Saudi Arabia, the consistent sedentary lifestyle and poor dietary habits are the reason for not just effecting work productivity but also pose a significant IDA-related economic burden on healthcare institutions[11]. With high prevalence rates, most of the cases require clinical management strategies such as transfusions and hospital stays. These add more burden and increase hospital utilization as well as expenditures[12,13].

Accurate diagnosis of IDA is reliant on biomarkers; however, they also face limitations in their diagnosis precision such as in studies where common blood iron biomarkers were compared against bone marrow biopsies which is a gold standard in IDA diagnostic only revealed ferritin to be the most specific (97-99% at a cutoff of 30 g/L) biomarker revealing marrow iron deficiency while it also demonstrated only moderate sensitivity (35-54%)[14]. Given the precision, bone marrow biopsies often are expensive, invasive and resisted to use in routine cases as it demands technical expertise and patient tolerance which only allow it to be used in secondary or tertiary care settings[15,16]. Serum ferritin levels ae another diagnostic criterion with values lower than 10-15 ng/mL considered to be 99% specificity for IDA, as they correspond to total body iron stores so they are sensitive and noninvasive test related to iron deficiency. However, ferritin being an acute-phase reactant is often elevated during infections or inflammations which masks iron

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deficiency making it less precise[16,17]. This makes ferritin sensitivity to drop from 92% to 45% in response to inflammatory conditions[18].

Mean Corpuscular Volume (MCV) being another important indicator for IDA diagnosis measures red blood cell size. MCV levels fluctuate in various situations, it decreases in microcytosis which correlates to late-stage iron deficiency, the decrease may also correspond to thalassemia while an increase is observed in Vitamin B12 deficiency, these lead to difficulties in interpretation[19,20]. Due to presence of thalassemia confounders it was reported that MCV <80 fL had 73% sensitivity for IDA but the specificity was reduced to 64%[21]. Another essential parameter for characterizing anemia is Hemoglobin, however it lacks specificity for iron deficiency as it only declines in case of depletion of iron stores[1]. Studies highlight that in cases of iron deficiency without anemia, patients have normal MCV along with other red cell parameters while serum ferritin levels were lowered. Comparatively in IDA patients, MCV was lower along with other red cell indices and similarly ferritin was also low. This indicates that ferritin is more sensitive in iron deficiency detection earlier than MCV changes start manifesting[22].

LITERATURE REVIEW

Iron Deficiency Anemia (IDA) is a critical global health challenge that affects one-third of the world's population significantly[23]. The pathophysiology of IDA initiates with disruptions in iron homeostasis exacerbating physiological needs exceeding available iron storage. Iron is responsible for various functions such as oxygen transport, DNA synthesis, and cellular energy production which require it to engage with hemoglobin and various enzymes[24,25]. IDA advances in three levels[26], first, without the onset of anemia, the ferritin levels are lowered signifying iron depletion, while this stage is often symptomatic some patients might report fatigue and diminished exercise tolerance[27]. Secondly, iron-restricted erythropoiesis develops which is characterized by increased soluble transferrin receptor (sTfR) and erythrocyte zinc protoporphyrin (ZPP) levels[28]. The final level leads to anemia which correlates with lowered hemoglobin levels often in conjunction with microcytosis and hypochromia[29]. IDA has consequences reaching far beyond hematological parameters neurological studies highlight that lower levels of iron deficiency can be detrimental for impairing cognitive function due to diminishing myelination or disrupted dopamine metabolism[30]. In terms of cardiovascular research, chronic IDA exerts increased cardiac output which may advance to heart failures in already vulnerable populations[31].

With the continuously evolving IDA diagnostic parameter landscape, there are still various clinical challenges. Serum ferritin is one of the most critical detectors of early iron deficiency which is capable of detecting depleted iron levels even lower than 30 ng/mL[32].. Because of its acute phase reactant nature, ferritin often confounds due to inflammatory conditions[33]. Its usage in conjunction with CRP-adjusted ferritin thresholds or collectively used with transferrin saturation has demonstrated potential for achieving improved diagnostic accuracy, especially when inflammations are involved[27,34]. MCV is another important diagnostic biomarker capable of detecting microcytic anemia in lower levels even below 80 fL, while it is confounded due to the presence of a-thalassemia trit in various populations[35]. This is being improved with the inventions of automated hematology analyzers measuring reticulocyte hemoglobin content (Ret-He) which is essential in the detection of real-time iron availability in cases of erythropoiesis[36]. Research further demonstrates Ret-He to be more preferred for for the detection of iron-restricted erythropoiesis with a sensitivity of 83.5% making it an early diagnostic indicator for the diagnosis of IDA[37]. With the emergence of new biomarkers more efficient in filling the diagnostic accuracy gap is hepcidin which declined during iron deficiency but increased in the presence of anemia[38,39]. In addition to these, the STfR-ferritin index is emerging to differentiate between IDA and inflammatory conditions which can diagnose the presence of anemia with a staggering specificity of 92.5%[40]. The challenge posed by these diagnostic parameters is their applications are restricted in a high burden IDA region with resource-limited settings.

The global distribution of IDA demonstrates wider disparities in Eastern and Western contexts, particularly within developing and developed nations. Western nations have a 5-8% prevalence in Western countries while Middle Eastern countries show 30% more burden in fe populations[3,11]. Studies in Saudi Arabia regarding pregnant women also underscore that 68.2% of rural regions pregnant women and adolescent girls meet the criteria for IDA diagnosis[41]. The economic impact of IDA can not be ignored due to the productivity losses it causes in developing countries[5]. Ork absenteeism and presenteeism is one dimension related to IDA that leads to economic impact and indirect cost burden which might correspond to approximately 1% of the Saudi Arabian GDP according to some analyses[42]. These economic losses are further compounded by the cognitive impacts of IDA which are demonstrated in scoring low scores which is indicative of reduced hemoglobin and iron levels leading to disrupting cognition in children and emphasizing the importance of iron during the developmental phase of cognition[43]. Middle East reports different management factors related to IDA management such as studies that report 45% of the Eastern population to be heterozygous for a-thalassemia which corresponds to increased carrier frequency which not only complicates microcytosis interpretation during clinical diagnosis but also misrepresents itself as genetic instead of nutritional factor[44]. Moreover, lower iron intake in the diet is a major risk factor for anemia, such as enhanced consumption of tea which has increased concentrations of polyphenols responsible for inhibition of iron absorption, there is also decreased intake of heme iron sources in diets like red meat and fish, all of these factors collectively contribute to increased anemia prevalence in population of Saudi Arabia[45].

Another dimension to these confounding factors is the sex-linked differences persistent in IDA which despite being well-established in research, are not widely understood. Women of reproductive age report a 21.4% prevalence of anemia as a result of menstrual loss which is a significant reason for iron deficiency and anemia, without accounting for inflammation or overweight status[46]. The central axis required for systemic iron regulation is the hepcidin-ferroportin here hepcidin is responsible for controlling iron absorption and release while estrogen modulates hepcidin presenting a hormonal layer to the overall pathway ultimately correlating reproductive hormones to iron metabolism and persistent sex difference of iron status[47,48]. Infants and children suffer microcytic anemia often due to iron deficiency highlighting the effect of an age-related factor in the presentation of IDA, while elderly patients report more normocytic anemic conditions mainly due to prevalent chronic diseases, renal impairment, or bone marrow disorders[49]. There was a distinct prevalence of 11.1% of anemia in a study of elderly patients along with mild normocytic anemia, low BMI, and poor dietary habits as frequent contributors. It is reflective that anemia prevalence in older patients is multidimensional comprising of nutritional deficiencies, chronic conditions, and comorbidities such as collagen diseases. This highlights that 30=50% of anemic elderly patients with overlapping medical conditions are caused by imperfect diagnosis and management of IDA[50].

Understanding the pathophysiology of IDA highlights crucial gaps in the understanding of the diagnostic parameters of IDA. Initially, the foundation of diagnostic parameters on mostly eastern thresholds

ignoring the basic difference in genetic, and lifestyle factors in addition to the thalassemic prevalence within the Saudi population challenges the biomarker performance[45]. There is no understanding present in the literature about the demographic variability in ferritin levels as described in a study of Jazan University students, reporting 27% lower ferritin levels in females than their male counterparts particularly due to menstrual losses[51] but there is not representation of MCV levels. Finally, the increased usage of PPI which is responsible for the conversion of dietary ferric into ferrous form is responsible for impairing iron metabolism increasing the risk of iron deficiency, and is linked frequently to IDA[52]. These factors challenge the diagnostic parameters while there is a lack of studies in the Saudi context regarding MCV-ferritin dynamics.

The role of physiological, epidemiological, and diagnostic factors exacerbated challenges in IDA, necessitates the understanding of the limitations of MCV and ferritin within clinical contexts. The high prevalence of IDA in Saudi Arabia in combination with its regional intricacies like thalassemia and dietary patterns highlights wider localized diagnostic approaches. This study seeks to evaluate the comparative utility of MCV and serum ferritin, two widely accessible biomarkers within the Saudi population, and the influence of demographic factors on their demographic accuracy.

The study aims to explore the following research questions:

1. How do MCV and serum ferritin levels perform as diagnostic biomarkers for IDA in a Saudi population, particularly in the context of high thalassemia prevalence?
2. What is the relationship between MCV and ferritin levels in patients with confirmed IDA?
3. How do demographic variables (age, sex) affect ferritin and MCV levels in this population?

The study is further guided by the following hypotheses:

- MCV and ferritin will exhibit an inverse correlation, consistent with iron-deficient erythropoiesis.
- Ferritin levels will be significantly lower in females and positively associated with age, reflecting lifecycle iron demands.

METHODOLOGY

Study Design: This study utilizes a cross-sectional design for examining the diagnostic capacity of mean corpuscular volume (MCV) and serum ferritin levels for identification of iron deficiency anemia (IDA). The research took place between July 2022 and July 2023 at the Human Clinic which is a specialized medicine and gastroenterology center located in Riyadh, Saudi Arabia. The dataset comprised of clinic patient's record, providing crucial and informative foundation for the assessment of biomarkers and IDA within general populations.

Sample Population: Sample population was recruited using non-probability convenience sampling while the sample size required was calculated to be 371 patients to achieve suitable statistical power. However, after the removal of participants with incomplete patient data and a through application of inclusion and exclusion criteria, 210 participants were deemed eligible for the final analysis. The sample majorly consisted of female patients (76.7%) which indicates higher prevalence of IDA among this demographic.

Inclusion Criteria: Patients that were 18 years or older confirmed diagnosis of IDA which is determined by hemoglobin (Hb) levels below 12 g/dL and serum ferritin concentrations under 100 ng/mL were included. Patients adhering to informed consent were part of the study.

Exclusion Criteria: Patients were excluded from the study if they had folate or vitamin B12 deficiency as these conditions could act as potential confounders and impact the results. Individuals receiving iron supplementation within 3 weeks prior to the study were also excluded to maintain baseline iron status measurement accuracy. Furthermore, to maintain the integrity of the data, participants diagnose with chronic inflammatory conditions, liver disease were not part of the study as these are known to disrupt ferritin levels leading to misrepresenting iron levels.

Data Collection: Data was collected from patient's electronic medical records including their demographic characteristics, medical history and laboratory results. Demographic information consisted of age, sex, and body mass index (BMI). Medical histories were scanned for chronic conditions like hypertension, diabetes and gastrointestinal disorders along with their medication usage such as proton pump inhibitors or supplements. Standardized clinical protocols were utilized for the measurement of laboratory parameters which involved MCV, hemoglobin levels, serum ferritin and iron concentrations.

Data Analysis: Data was analyzed using statistical analyses with the help of SPSS package. Descriptive statistics were employed for characterization of demographic and clinical dimension of the study population. Whereas Inferential statistics such as Pearson's correlation tests were used to identify the relationship between key variables including MCV, ferritin, HB level, age and sex. Moreover, Independent t-test and chi square were also used for the comparative analysis and logistic regression analysis was used to determine potential risk factors for IDA. Significance of statistic values was determined with a p-value of less than 0.05.

Ethical Consideration: Compliance with ethical standards was ensured with the approval of study protocol from the Institutional Review Board (IRB) of Imam Mohammed bin Saud Islamic University (IMSIU). Participants were provided with informed consent to ensure privacy and data safety. Further confidentiality was maintained with restricted access to participants' information to authorized personnel only. Additionally, the study also adhered to the Declaration of Helsinki which promotes patient welfare and data protection through the course of study.

RESULTS

Demographic Characteristics

The study assessed 210 patients meeting the inclusion criteria, while the demographic distribution demonstrated a wider disparity between gender representation (Table 1). Most participants (76.7%) were female while only 23.3% were male. The mean age of study population, 38.44 years revealed a significantly younger and middle-aged group. Body Mass Index was reported to be alarmingly high with a mean of 41.11 kg/m2 (SD = 189.4).

Table 1. Demographic Characteristics of the Study Population

Gender	Frequency	Percentage
Male	49	23.3 %
Female	161	76.7 %
	Mean (SD)	
Age	38.44 (15.13) years	
BMI	41.11 (189.4) kg/m²	

Descriptive Statistics

Laboratory Parameters

There were stark differences within hematological parameters as highlighted by the laboratory data (Table 2). With a SD = 68.94, the

mean serum iron level was 104.51 mg/dL with a vast range from 9.7 to 347.2 mg/dL (n = 129). Meanwhile ferritin levels within the population had a mean of 61.81 ng/mL ranging from 0.20 to 594.00 ng/mL. The descriptive data further highlights that the average MCV was 81.84 fL (SD = 10.78) and Hemoglobin (Hb) levels report average values 13.66 g/dL (SD = 6.35), while the broad range of Hemoglobin 6.50 to 82.70 g/dL is noteworthy.

Table 2. Descriptive Statistics of Laboratory Results

Variables	N	Minimum	Maximum	Mean (SD)	Range
Iron	129	9.7	347.20	104.50 (68.93)	9.7–347.20 µg/dL
Ferritin	210	.20	594.00	61.81 (85.88)	0.20– 594.00 ng/mL
MCV	210	11.10	96.00	81.83 (10.77)	11.10–96.00 fL
Hgb	210	6.50	82.70	13.65 (6.34)	6.50–82.70 g/dL

Medical Conditions and Medical Use

The descriptive analysis of comorbidities highlights a significant portion of participants (67.2%) had no chronic medical conditions (Figure 1). Patients with prevalent chronic comorbidities were diabetes mellitus (8.5%) and hypertension (8.0%), whereas other conditions comprised of gastrointestinal tract disorders (10.4%), hyperthyroidism (6.5%), asthma (3.0%) and lowered vitamin D levels (2.0%) were present in lower frequencies. Furthermore, analysis of medication data demonstrated a larger distribution (32%) of patients without any consistent use of medication at the time of evaluation. However, patients with prescribed medication regimen reported using proton pump inhibitors (PPIs) were the most common (35.4%), flowed closely by gastrointestinal medications (29.6%) and vitamin D supplements (17.0%). Another striking finding was the reduced use of iron supplements (5.3%) which might reflect patients being unaware of their iron status or not seeking treatment (Figure 2).

Inferential Analysis

Investigation of interlinking between demographic variables such as age, gender, BMI and laboratory identifiers of iron status like iron, ferritin, MCV and Hb were analyzed using Pearson correlational analysis while aligning it with the study's research hypotheses (Table 3).

Hypothesis 1: An Inverse Correlation Exists Between MCV and Ferritin

Correlation between MCV and ferritin level was reported to be significantly negative ($r = -0.337$, $p < 0.001$), which proves the hypothesis statistically right, highlighting that ferritin levels decline identifying iron depletion whereas MCV has increased. These findings are typical representations of pathophysiological patterns in IDA, which often represent iron deficiency resulting in microcytic erythrocyte

Table 3. Correlations Between Demographic and Laboratory Variables

Variables	Tests	Age	Gender	BMI	Iron	Ferritin	MCV	Hgb
Age	Pearson Correlation	1	-0.044	0.043	0.039	0.182**	-0.022	-0.040
	Sig. (2-tailed)	---	0.524	0.563	0.660	0.008	0.747	0.567
	N	210	210	181	129	209	210	210
Gender	Pearson Correlation	-0.044	1	0.029	0.001	-0.460**	-0.014	-0.158*
	Sig. (2-tailed)	0.524	---	0.703	0.988	0.000	0.837	0.022
	N	210	210	181	129	209	210	210
BMI	Pearson Correlation	0.043	0.029	1	-0.119	-0.047	-0.145	-0.064
	Sig. (2-tailed)	0.563	0.703	---	0.214	0.530	0.052	0.390
	N	181	181	181	111	180	181	181
Iron	Pearson Correlation	0.039	0.001	-0.119	1	0.110	0.029	0.093
	Sig. (2-tailed)	0.660	0.988	0.214	---	0.218	0.746	0.293
	N	129	129	111	129	128	129	129
Ferritin	Pearson Correlation	0.182**	-0.460**	-0.047	0.110	1	0.005	0.337**
	Sig. (2-tailed)	0.008	0.000	0.530	0.218	---	0.943	0.000
	N	209	209	180	128	209	209	209
MCV	Pearson Correlation	-0.022	-0.014	-0.145	0.029	0.005	1	-0.487**
	Sig. (2-tailed)	0.747	0.837	0.052	0.746	0.943	---	0.000
	N	210	210	181	129	209	210	210
Hgb	Pearson Correlation	-0.040	-0.158*	-0.064	0.093	0.337**	-0.487**	1
	Sig. (2-tailed)	0.567	0.022	0.390	0.293	0.000	0.000	---
	N	210	210	181	129	209	210	210

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

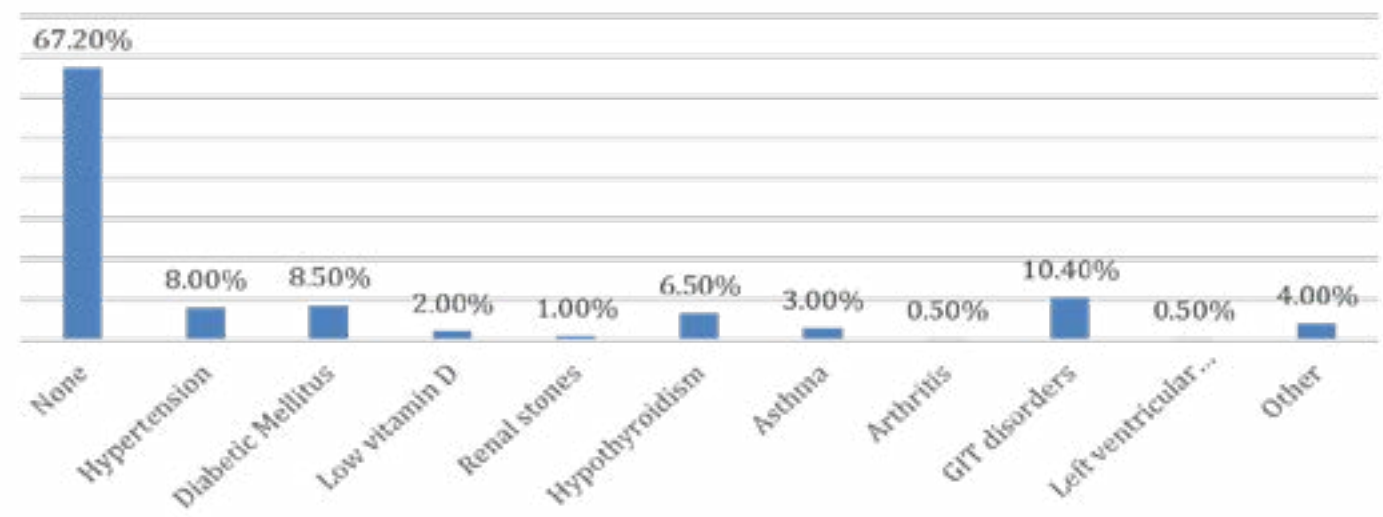

Figure 1. Incidence Rates of Medical Conditions Among Study Participants

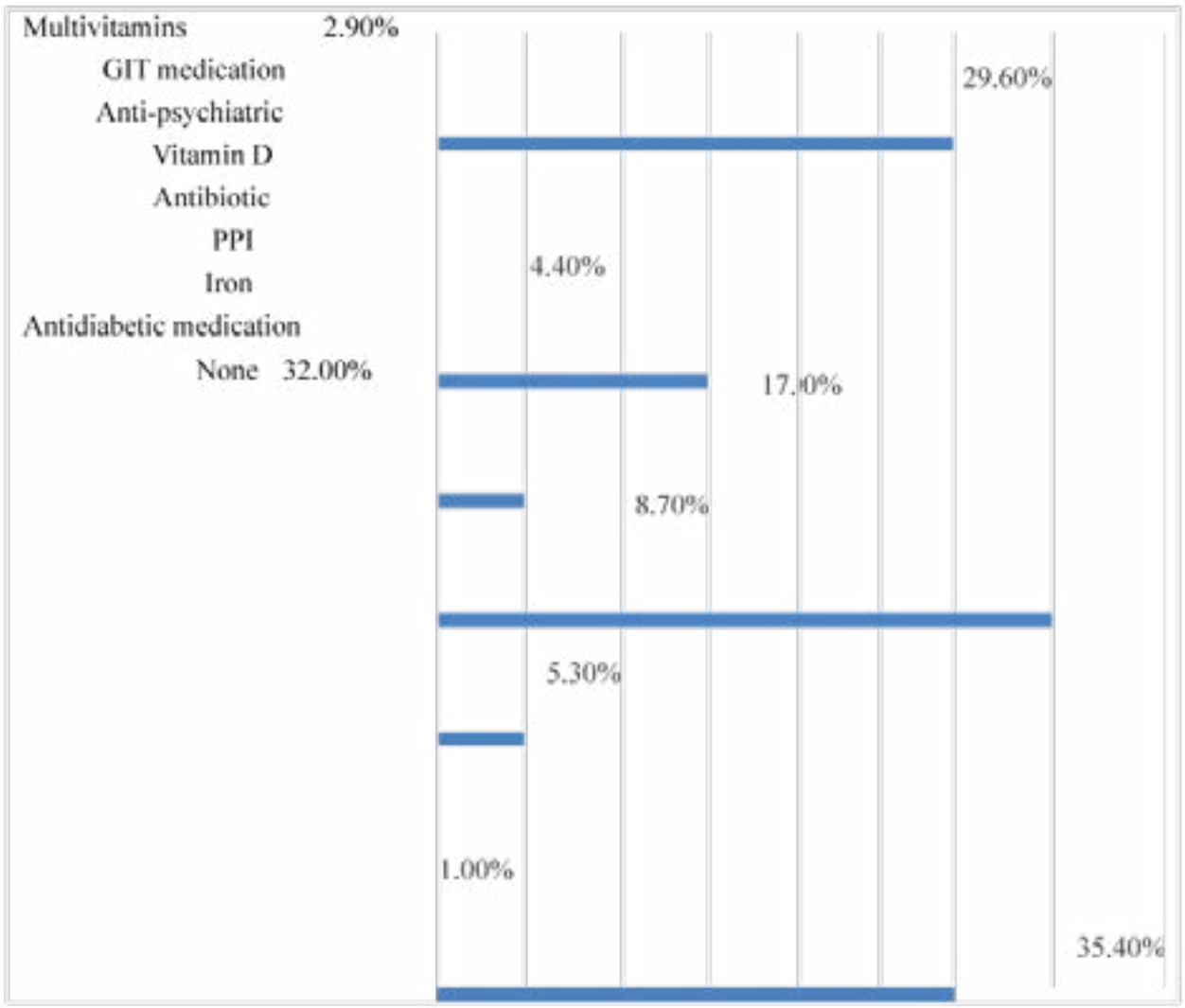

Figure 2. Medications Reported by Study Participants at the time of Evaluation

development. It is striking to note that MCV also demonstrate an inverse correlation with regards to hemoglobin levels ($r = -0.487$, $P < 0.001$). This reflects that MCV does not only report iron status but also play critical role in identifying the clinical severity of anemia, which increases its diagnostic relevance. Notably, there was no significant correlation between MCV and demographic variables such as age ($r = -0.22$, $p = 0.747$), sex ($r = -0.014$, $p = 0.837$ and BMI ($r = -0.145$, $p = 0.052$). These findings underscore that demographic variables are not responsible for confounding MCV values.

Hypothesis 2: Ferritin Levels will be Lower in Females and Positively Associated with Age

Another significant correlation was reported between ferritin levels and age ($r = 0.182$, $p = 0.008$) which support the second hypothesis, demonstrating that ferritin concentrations are often observed to be higher within older populations. It also aligns with existing literature that describe the accumulation of iron with increasing age gradually, particularly reflective in postmenopausal women who are not experiencing and menstrual blood loss anymore. Furthermore, a strong inverse correlation between ferritin and gender ($r = -0.460$, $p < 0.001$) was observed where women exhibited notable lower ferritin levels against their male counterparts. This reflects a global epidemiological phenomenon where women of reproductive age are subjected to be disproportionately impacted by IDA because of increased iron requirements of the body in pregnancy and menstruation. The sex-based disparities related to iron storage capacity highlights the need to classify iron deficiency risk assessment by gender.

Moreover, the results indicate some other critical correlational findings where a strong positive correlation ($r = 0.337$, $p < 0.001$) was observed among Hemoglobin and ferritin levels which supports interconnecting clinical roles of them as iron stores and oxygen-carrying capacities. A slightly weak but notable negative statistical correlation was also observed between gender and hemoglobin levels ($r = -0.158$, $p = 0.022$) which again suggests depleted hemoglobin levels within females. However, there were no significant correlations reported between BMI and iron biomarkers ($p > 0.05$) which highlights limited compounding influence over variables within this demographic.

Summary of Hypothesis Testing

The inverse correlation confirmed between MCV and ferritin levels is crucial to the persistent diagnostic challenges in IDA among the Saudi population which typically has an increasing burden of high a-thalassemia carrier levels along with widespread use of PPI often leading to confounding traditional markers. Despite these confounders, the correlational strength identified in the findings between MCV, and ferritin are reflective of the importance of diagnostic values these biomarkers still retain. However, it is imperative to note that even with the moderate magnitude of the correlation ($r = -0.337$) is indictive of not using either marker for definitive diagnosis in isolation. Additionally, the interdependence of iron stores and erythropoiesis was highlighted by the strong correlation existing between ferritin and hemoglobin. Ferritins sensitivity for the early detection of iron deficiency is uprooted by the fact that ferritin levels drop even before the decline of hemoglobin, whereas MCV is responsible for demonstrating advanced cellular level modifications happening in erythrocyte morphology.

DISCUSSION

Iron deficiency anemia being one of the most pressing public health challenges particularly in regions where prevalence rates are exacerbated mostly due to sociodemographic and clinical patterns such as Middle Eastern countries, namely Saudi Arabia. This study explored and compared the two of the widely available biomarkers i.e. MCV and serum ferritin for diagnostic performance evaluation in the detection of IDA. It also investigated the influence of various demographic factors such as age and sex on these diagnostic parameters.

Findings confirmed the diagnostic performance of both biomarkers, with a staggering statistically significant inverse correlation ($r = -0.337$, $p < 0.001$) observed between MCV and serum ferritin levels highlighting their efficiency in pointing out IDA. These findings aligns strongly with the literature that describes ferritin being an iron storage protein faces decline during iron deficiency while MCV drops due to the presence of microcytosis during the later stages of iron depletion[53,54]. Given the diagnostic reliability, both biomarkers face various limitations that jeopardizes their individual diagnostic efficiency. Non-iron-related causes of microcytosis often act as confounders to MCV, particularly in instances of thalassemia trait presence or chronic inflammatory states[55,56]. In regions where a-thalassemia trait is prevalent such as Saudi Arabia, the independent presence of microcytosis to IDA is very common, which results in false-positive interpretation when the diagnosis are solely reliant on MCV levels[57]. On the contrary, a relatively sensitive and specific biomarker of iron deficiency is serum ferritin, but its ability to act as an acute-phase reactant turns out to be a major limitation. Falsely elevated ferritin levels during the presence of an inflammatory condition are responsible to conceal iron deficiency[58]. These findings provide a deeper understanding that both MCV and ferritin are useful biomarkers individually, however, none of them can be used alone for a definitive diagnosis, particularly in a distinguished population with unique genetic and clinical confounders. Additionally, alternative diagnostic approaches such as Reticulocyte Hemoglobin Equivalent (Ret-He) shows increased promising potential in the identification of functional iron deficiency while soluble transferrin receptor (sTfR) is efficient against differentiation of IDA from anemia of chronic disease[59,60]. Utilizing these biomarkers in a diverse clinical setting like Saudi Arabia may provide required diagnostic specificity and lower the false positive burden.

An inverse correlation ($r = -0.337$) was confirmed in the findings between MCV and ferritin levels which are reflective of the pathophysiological routes of IDA. The decline of iron stores as reflected by decreased ferritin level, are often followed by the presence of microcytic red blood cells as reflected by lowered MCV and finally iron becomes scarce for successful erythropoiesis. These findings are in agreement with previous pathophysiological studies underscoring the decline of serum ferritin levels to be superceded by hematological changes[61]. This gradual advancement emphasizes the roles of these biomarkers in different stages, while MCV is efficient for advanced diagnosis of iron depletion, ferritin contrarily serve as an early indicator. This highlights an important aspect of utilizing a two-step clinical diagnosis strategy where for the early detection ferritin levels are investigated and for the confirmation of progression towards anemia, MCV is ordered.

Another important aspect of the findings were the impact of demographic factors on IDA biomarkers which underscores wider differences in gender and age. Lowwer ferritin levels were observed in the female population in comparison to males ($r = -0.460$, $p < 0.001$) while positive associations with age ($r = 0.182$, $p = 0.008$) were reported in the findings. These findings also corroborate the global data that underscores women of particularly reproductive age are more inclined towards an increased IDA risk due to factors like menstrual blood loss, pregnancy and lactation[62]. While estrogen's regulatory effects are also responsible to influence hepicidin and iron homeostasis, collectively adding to complexity of women's iron metabolism[63]. Moreover, the positive correlation among age and ferritin in women particulalrly after the cesation of menstruation leads to iron loss and

increased accumulation of ferritin. Whereas, normocytic anemia related to chronic diseases in older populations are more prevalent[64]. These findings highlight careful characterization between IDA and other etiologies in elderly populations. An intresting finding to note is no correlation was proven statistically significant between BMI and MCV or ferritin during the study which is contradictory to some studies that highlights obesity induced inflammation to influence ferritin levels[65]. This might be explained due to the exclusion of patients with existing inflammatory disorders, that led to negligible observance of confounding effect of inflammation within present study sample.

Additionally, a secondary finding observed as the increased use of proton pump inhibitors (35.4%) in the sample population. PPI's which are often prescribed in gastrointestinal disorders which inhibit gastric acid secretion that is crucial for converting dietary ferric into ferrous iron, ultimately challenging iron absorption[66]. Various studies relate increased risk of IDA to the continous use of PPI (52). However, in the Saudi population, this correlation is critical due to the prevalence of dietary patterns and H. pylori infections[67], which collectively adds to the impairment of iron absorption. These findings highlight that healthcre providers should be cautious while prescribing PPIs particulalry in the context of high risk iron deficiency populations. Alternative therapies and nutritional counseling should be adopted advocating for heme iron sources and iron absorption enhancers should be implemented into care strategies. The diagnostic limitations observed in this study demonstrate both MCV and ferritin to have limitations particularly in the Saudi Arabian context where there is high genetic heterogeneity and PPI use hich demands for newer more comrehensive diagnostic protocols. Using MCV and Ferritin together with additional tests such as CRP-adjusted ferritin, sTfR and Ret-He could improve overall diagnostic accuracy. This also calls for localized reference ranges along ith diagnostic thresholds that are parallel to demographic and genetic variability of the specific population.

Study Limitation

Study sample limitations such as dominancy of female prticipants (76.7%) may limit the generalizbility of the findings in general demographics or against male counterparts. Employing convenience sampling may intorduce potential selection bias which is reflective of external validity. The study only includes data from a single clinic in Riyadh hich is indictive of not capturing enough regional or national variability.

Future Recommenations

Research studies should focus on desigining larger, multicentric studies including diversified Saudi subpopulations for validity of regional thresholds required for MCV/ferritin diagnostic criteria. Using alternative diagnostic criteria for the improvement of diagnostic accuracy in st risk population ith thalessemia traits or long-term usage of PPI.

CONCLUSION

The study highlight the interlinking of both biomarkers of IDA, MCV and ferritin presents an inverse correlation ($r = -0.337$, $p < 0.001$) indicating iron-deificent erythropoiesis. Demographic findings underscores decreased ferritin levels in females ($r = -0.460$) and positive relationship ith age ($r = 0.182$). All these findings reflect alignment with international trends emphasizing on sex-specific iron demands and the accumulation of iron within postmenopausal omen. However, presentation of increased thalessemia traits and utilization of PPI in Saudi Arabia is the reason for challenges observed in biomrker interpretation, warranting caution from clinicl providers. Despite the efficacy of early detection through ferritin and advanced progression through MCV are critical indicators, their individual efficacy is negligible due to the presence of consistent confounders. The study recommends integration of CRP-adjusted ferritin, reticulocyte indices and general thereshold values for ehnacing the accuracy of diagnostic criterias. It is also imperative to address PPI-related iron malabsorption along with healthy dietary habits to combat IDA burden.

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