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Iron Status Among Women of Reproductive Age in Tertiary Health Center: A Cross-Sectional Study

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Abstract

Background: Iron deficiency anaemia is the most common type in women of the reproductive age group (15-49 years old). A low iron profile status is diagnostic of iron deficiency, which can lead to anaemia. Present study aimed to perform iron profiling in women within the reproductive age group assess their iron status and insight into their risk of iron deficiency or iron overload in reproductive-age women.

Methods: serum was collected from recruited study participants, serum Iron, Total iron binding capacity(TIBC), Serum Ferritin, hemoglobin level, Transferrin saturation, glucose and TSH levels were estimated.

Results: The study assessed the iron profile in 138 women aged 18-38 years, with an average age of 20.507 years. The findings showed normal average glucose and TSH levels, indicating no significant glucose metabolism or thyroid issues. Iron parameters (iron, TIBC, UIBC, transferrin saturation) decreased with age, while ferritin levels increased. The average TIBC was 521.698 µg/mL, with 84.8% above the normal range, suggesting potential iron deficiency despite elevated TIBC and iron levels. UIBC and transferrin saturation also indicated iron imbalances. Correlations showed higher TSH, glucose, and ferritin levels with age, and inverse relationships between iron and UIBC, and ferritin and TIBC. The median ferritin level was 14.239 ng/mL, with 34.1% having depleted iron stores. Serum iron levels averaged 178.529 µg/mL, with 62.3% above normal. The average hemoglobin level was 12.091 g/dL, with 37.7% indicating anemia.

Conclusion: The study highlights significant variability in iron status among reproductive-aged women, emphasizing the need for targeted nutritional and medical interventions.

Keywords: iron, anemia, ferritin, TIBC

INTRODUCTION:

Iron is the most commonly found transition metal in living cells. The average human requires 20 mg of elemental iron/day to produce enough erythrocytes (red blood cells). In addition to erythropoiesis, iron is essential for several biological activities within all cells, such as energy production, DNA replication, and repair. Because of its ability to reversibly gain or lose single electrons by transiting between distinct oxidation states, iron is required for a wide range of critical enzymatic redox processes within cells(Ng et al., 2019).

Iron deficiency (ID) is a condition with insufficient iron to support the proper physiological function of tissues(Mawani & Aziz Ali, 2016). iron deficiency without anaemia is achievable before haemoglobin (Hb) concentrations fall below the threshold for the individual sex and age group(Williams et al., 2023). Iron insufficiency is a global issue affecting both developed and underdeveloped countries. Anaemia, with or without iron deficiency, is a frequent condition globally(Hasdiana, 2018).

Anaemia is a condition in which not enough healthy red blood cells are present within the blood, which can lead to fatigue, pallor, shortness of breath, dizziness, etc. Iron deficiency anaemia is the most common type in women of the reproductive age group (15-49 years old). iron profile status is assessed to determine whether someone suffers from iron deficiency. A low iron profile status is diagnostic of iron deficiency, which can lead to anaemia. As of 2019, the World Health Organization (WHO) estimated that about 30% of women have anaemia globally, and 50% of these cases occur due to iron deficiency(Hasdiana, 2018). WHO has also noted the increased prevalence of anaemia in developing countries like India. Based on a study done in 2019, about 66.4% of women (15-49 years old) suffer from anaemia in India alone(Gautam et al., 2023). Iron is an essential micronutrient required by our body. Thus, an improper diet can result in iron deficiency. It can be found in red meat, cereals, green leafy vegetables, poultry, fish, etc. and has vital functions within the human body. Iron is the main component of haemoglobin. During menstruation, a woman will lose blood for an average of 4 to 5 days, depleting red blood cells(Gautam et al., 2023). Due to the increased loss of blood cells during menstruation, the body will begin to use the iron stores to make more blood cells. This will result in an increased need for iron leading to iron deficiency anaemia. Therefore, women are more prone to iron deficiency anaemia when compared to men(Gautam et al., 2023). This can result in increased fainting episodes, pallor, etc., eventually leading to more severe health-related concerns. During pregnancy, the fetus's growth and development require a large amount of iron. Surprisingly, epidemiologic studies have found that excessive iron

intake or high iron status can be harmful to pregnancy and are linked to reproductive diseases ranging from endometriosis to preeclampsia (1).

Iron excess causes a wide range of functional alterations in the human body. Excess iron deposits are primarily found in the liver, heart, and endocrine organs. Damage to the liver can cause chronic liver disease, cirrhosis, and hepatocellular cancer. Damage to the heart muscle can result in cardiac failure and irregular heartbeat. Pancreatic damage can cause high blood glucose levels and "bronze" diabetes. Hypothyroidism and hypogonadism can cause fatigue, hair loss, infertility, and diminished libido. Joint involvement causes arthritis. Neurological involvement can hasten neurodegenerative disorders like Alzheimer's disease(Zeidan et al., 2021). Serum ferritin levels greater than 300 ng/ml in males and 150 to 200 ng/ml in menstrual females may suggest iron excess. However, serum ferritin levels can rise for various reasons, including inflammation, infection, and liver illness. Ferritin is referred to as an acute-phase reactant(Auerbach, 2023). Total binding iron capacity (TIBC) may also be within normal limits. Fasting and a serum transferrin saturation percentage of more than 45% can help with further diagnosis(Faruqi et al., 2024).

Women must maintain a normal iron profile status because it can lead to multiple health complications. Low iron levels can lead to an increased risk of neurodevelopmental disorders like autism and can cause miscarriages as blood helps carry oxygen to the foetus [5]. Whereas, Iron overload causes a variety of issues that can have a serious influence on a person's health. Excessive iron accumulation in important organs, particularly the liver, heart, and endocrine glands, can cause organ damage by producing reactive oxygen species. Complications can include liver diseases like cirrhosis and hepatocellular carcinoma, cardiomyopathies that affect the heart, and endocrine disorders including diabetes and hypothyroidism. Furthermore, iron excess has been related to increased susceptibility to infections and a weakened immune response(Zeidan et al., 2021).

While numerous studies have documented the prevalence and consequences of iron deficiency and iron overload, there is a need for comprehensive iron profiling specifically among women of of reproductive age in India, which has high anaemia rates. Previous research has predominantly focused on the general population or specific subgroups without evaluating the full spectrum of iron status within this demographic. Therefore, in the present study, we aimed to perform iron profiling in women within the reproductive age group, which may provide evidence of their iron status and insight into their risk of iron deficiency or iron overload in reproductive-age women.

METHODOLOGY:

DATA COLLECTION: In the present Cross-sectional study, 138 Healthy volunteers visiting Justice KS Hegde Charitable Hospital who were willing to participate were recruited for the study. The study was conducted after obtaining the ethical clearance from the Institutional ethics committee [INST.EC/EC/056/2023]. The subjects were recruited only after receiving written informed consent. Women of reproductive age group (between the ages of 15 – 45 years) were included in the study. Participants already on iron supplementation therapy were excluded from the study. The baseline and demographic data were collected by the questionnaire method

BIOCHEMICAL ESTIMATION:

3ml of venous blood was collected using the standard clinical procedure in the plain vacutainers. Serum separated was stored at -80°C until further use.

serum Iron: Serum iron was estimated using commercially available colorimetric kit method (Agappe diagnostics ltd, India). In an acidic medium, ferric iron dissociated from transferrin, carrier protein, and reduced to ferrous form. Ferrous ion complex with a sensitive iron indicator chromogen, resulting in the formation of intensely colored chromophores which is measured at 630nm spectrophotometrically. The color intensity of this color is directly proportional to the concentration of iron present(Sidel et al., 1984). Reference value is 37-145µg/dL in females.

Total iron binding capacity: Total iron binding capacity (TIBC) was estimated using commercially available colorimetric kit method (Agappe diagnostics ltd, India). An acidic buffer containing iron-binding dye and ferric chloride is added to the serum, which releases iron from transferrin and forms colored complex. Then a neutral buffer is added which shifts the pH, enhancing the affinity of transferrin for iron. This allows transferrin to iron from the dye-iron complex, leading to a decreased absorbance directly absorbance directly proportional to serum total iron binding capacity. The absorbance in measured at 630nm spectrophotometrically. Reference value is 250-450µg/dL (Burtis & Bruns, 2008)

Serum Ferritin: Serum Ferritin was estimated using a commercially available ELISA kit (Calbiotech, California), following the method mentioned in the procedure booklet received along with the kit. Recommended range is 10-200ng/mL(Polson et al., 1988).

Hemoglobin: The hemoglobin level and total cell count will be performed using a cell counter.

Transferrin saturation: Transferrin saturation (%) was calculated using the formula, Serum Iron/ Serum TIBC x100 (Gebreegziabher & Stoecker, 2017)

STATISTICAL ANALYSIS: The data was compiled using SPSS software. Continuous variables were expressed in terms of mean and standard deviation and analyzed using student t-test. Non-parametric values were expressed in median and interquartile range. Categorical data was expressed as percentage and analyzed using chi-square. Associations between iron profile and continuous variables were assessed using Pearson correlations $p \leq 0.05$ was considered statistical significance.

RESULTS

The study involved 138 reproductive aged female participants aged between 18-38 with the mean of 20.507±4.596years. 67.4% of the recruits were below the age of 20, 26.1% were between the age of 20-30 and 6.5% were above the age of 30. The biochemical characteristics of the study participants is shown in table 1. The mean random glucose, serum TSH of the recruited participants was 96.367±10.676mg/dL and 1.829±0.937 µIU/mL respectively. The average Hb was 12.091±1.159g/dL out of which 37.7% of the partcipants has low Hb level and 62.3% had Hb within normal range. The overall serum iron level was above the normal range of 37-145µg/mL with the average of 178.529±65.764µg/mL. 37.7% of the female had normal serum iron level whereas 62.3% of the female had higher than normal serum iron. The median serum ferritin was 14.239 ng/mL with interquartile range of 6.968-24.571 ng/mL. 34.1% had serum ferritin level of <10 ng/mL whereas 65.9% had with the normal range (10-200 ng/mL). the average TIBC, 521.698±127.129 µg/mL, was above the normal range (250-400 µg/mL). 2.2% showed <250 µg/mL 13.0% showed between 250-400 µg/mL and 84.8% showed >400 µg/mL of serum TIBC, the average UIBC was 344.535±136.554µg/mL of which 8.0% presented <160 µg/mL, 43.5% presented 160-360 µg/mL and 48.6% presented >360 µg/mL of serum UIBC. The average Transferrin saturation was observed to be 32.655(25.780-44.403% in which 7.2% ,79.7% and 13% of the study participants presented <15%, 15-50% ad >50% Transferrin saturation respectively.

Table 1: descriptive characteristics of the study participants(n=138)

	n(%) or mean±SD
Age (years)	20.507±4.596
<20 years	93(67.4)
20-30 years	36(26.1)
>30 years	9(6.5)
Random Glucose (mg/dL)	96.367±10.676

Hb(g/dL)	12.091±1.159
<12g/dL	52(37.7)
12-15g/dL	86(62.3)
>15g/dL	0(0)
Serum TSH(μIU/mL)	1.829±0.937
Serum Iron (μg/mL)	178.529±65.764
<37 μg/mL	0(0)
37-145 μg/mL	52(37.7)
>145 μg/mL	86(62.3)
Serum ferritin(ng/mL)	14.239(6.968-24.571)
<10 ng/mL	47(34.1)
10-200 ng/mL	91(65.9)
>200 ng/mL	0(0)
TIBC (μg/mL)	521.698±127.129
<250 μg/mL	3(2.2)
250-400 μg/mL	18(13.0)
>400 μg/mL	117(84.8)
UIBC (μg/mL)	344.535±136.554
<160 μg/mL	11(8.0)
160-360 μg/mL	60(43.5)
>360 μg/mL	67(48.6)
Transferrin saturation(%)	32.655(25.780-44.403)
<15%	10(7.2)
15-50%	110(79.7)
>50%	18(13)

Table 1 Data presented as n(%) for categorical variables and mean ±SD or median[interquartile range] unless indicated otherwise for continuous variables. Hb, hemoglobin; TSH, thyroid stimulating hormone; TIBC, Total iron binding capacity; UIBC, unconjugated iron binding capacity.

Correlation matrix of the study parameters(table 2) showed a significant association between iron profile, Hb, random glucose, TSH and age. A significant negative association was observed between age and iron (r=-0.242, p=0.004), TIBC (r=-0.362, p=<0.001), UIBC (r=-

0.225, p=0.008) and a positive significant correlation was observed between age and TSH (r=0.174, p=0.041), random glucose (r=0.260, p=0.002) and ferritin (r=0.272, p=0.001). Iron positively correlated with serum TSH (r=0.210, p=0.013), transferrin saturation (r=0.712, p=<0.001) and negatively correlated with UIBC (r=-0.416, p=<0.001). No strong association was observed between glucose and iron profile. Ferritin positively associated with Hb level (r=**0.463, p=<0.001)** whereas negatively associated with TIBC (r=**-0.189, p=0.026)** and UIBC (r=**-0.149, p=0.081)**. association between age and the biochemical characteristics of the study participants presented as linear regression model in figure 1.

Table 2: association between age, hemoglobin, TSH, random glucose and iron profile in reproductive aged women

		Age	Serum TSH	Random glucose	Hb	Ferritin	Iron	TIBC	UIBC	Transferrin saturation
Age	r p	1	**0.174** **0.041**	**0.260** **0.002**	0.044 0.609	**0.272** **0.001**	**-0.242** **0.004**	**-0.362** **<0.001**	**-0.225** **0.008**	-0.025 0.770
Serum TSH	r p		1	0.098 0.257	-0.061 0.478	-0.004 0.966	**0.210** **0.013**	-0.010 0.904	-0.107 0.211	**0.173** **0.042**
Random glucose	r p			1	-0.141 0.103	0.102 0.236	0.047 0.587	-0.078 0.364	-0.090 0.300	0.127 0.140
Hb	r p				1	**0.463** **<0.001**	0.052 0.547	**-0.226** **0.008**	**-0.226** **0.008**	**0.220** **0.009**
Ferritin	r p					1	-0.073 0.396	**-0.189** **0.026**	**-0.149** **0.081**	0.025 0.769
Iron	r p						1	0.059 0.489	**-0.416** **<0.001**	**0.712** **<0.001**
TIBC	r p							1	**0.881** **<0.001**	**-0.553** **<0.001**

UIBC	r p								1	**-0.817** **<0.001**
Transferrin saturation	r p									1

Table 2 association of haemoglobin, TSH, random glucose, age and iron profile presented as Pearsons's correlation matrix. r-Pearsons's correlation coefficient. P-value less than 0.05, 0.001 considered significant. Hb, hemoglobin; TSH, thyroid stimulating hormone; TIBC, Total iron binding capacity; UIBC, unconjugated iron binding capacity.

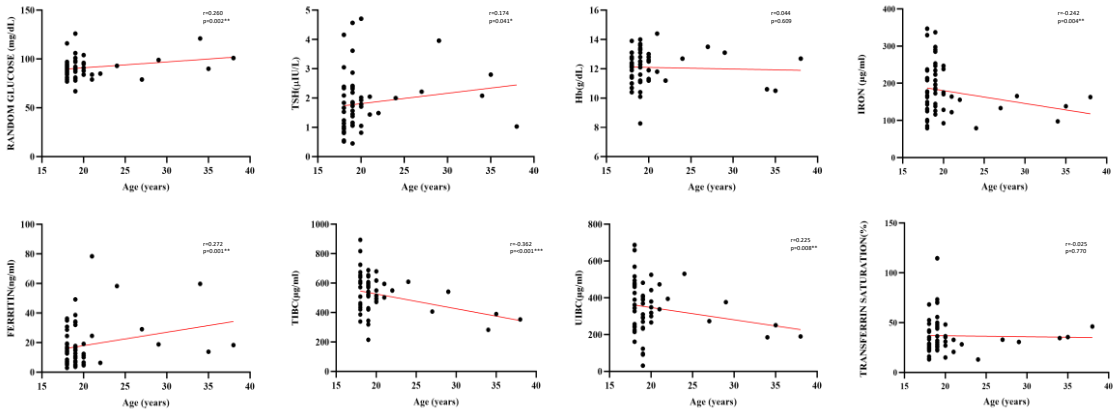

*Figure 1 Correlation of random glucose, TSH, Hb and iron profile with age. r-Pearson correlation coefficient. *p<0.05, **p<0.01, ***p<0.001. Hb, hemoglobin; TSH, thyroid stimulating hormone; TIBC, Total iron binding capacity; UIBC, unconjugated iron binding capacity.*

DISCUSSION

The present study demonstrated iron profile in reproductive aged women. The study involved 138 women of reproductive age, ranging from 18 to 38 years old, with an average age of 20.507±4.596 years. A significant majority (67.4%) were younger than 20 years, 26.1% were between 20 and 30 years, and only 6.5% were older than 30 years. The average random glucose level and serum Thyroid Stimulating Hormone (TSH) level within the normal range, indicating no significant issues with glucose metabolism and suggesting normal thyroid function in most participants.

The present study reported iron, TIBC and UIBC overload in reproductive aged women. Serum ferritin level was observed within range with 34.1% cases being lower than normal. The current study demonstrates the biomarkers of iron status, iron, TIBC, UIBC and transferrin saturation decrease with age whereas ferritin increased with age. The average TIBC was 521.698±127.129 µg/mL, which is above the normal range (250-400 µg/mL). Only 2.2% had TIBC below 250 µg/mL, 13.0% were within the normal range, and a vast majority (84.8%) had TIBC above 400 µg/mL. Elevated TIBC can indicate iron deficiency, as the body increases its capacity to bind iron when iron stores are low. However, the current study demonstrated elevated levels of TIBC and iron contradicting the earlier studies(Johnson Wimbley & Graham, 2011)

The average UIBC was 344.535±136.554 µg/mL, with 8.0% below 160 µg/mL, 43.5% within 160-360 µg/mL, and 48.6% above 360 µg/mL. UIBC, like TIBC, can indicate iron deficiency when elevated, suggesting that many participants may be experiencing an imbalance in iron homeostasis(Johnson Wimbley & Graham, 2011). The average transferrin saturation was 32.655%, with 7.2% below 15%, 79.7% within the normal range (15-50%), and 13.0% above 50%. Transferrin saturation reflects the percentage of transferrin that is saturated with iron, and deviations from the normal range can indicate disorders of iron metabolism(McLaren et al., 2001).

Negative correlation between age and iron ($r=-0.242$, $p=0.004$), TIBC ($r=-0.362$, $p<0.001$), and UIBC ($r=-0.225$, $p=0.008$) suggests that as age increases, these iron parameters decrease, indicating potential changes in iron metabolism with age(Zeidan et al., 2021). Positive correlation between age and TSH ($r=0.174$, $p=0.041$), random glucose ($r=0.260$, $p=0.002$), and ferritin ($r=0.272$, $p=0.001$) suggests that older participants tend to have higher TSH, glucose, and ferritin levels, potentially reflecting age-related changes in thyroid function, glucose metabolism, and iron stores. Positive correlation between iron and TSH ($r=0.210$, $p=0.013$) and transferrin saturation ($r=0.712$, $p<0.001$) indicates that higher iron levels are associated with higher TSH and transferrin saturation.

Negative correlation between iron and UIBC ($r=-0.416$, $p<0.001$) reflects the inverse relationship where higher iron levels correspond to lower UIBC, consistent with expected physiological responses. Positive correlation between ferritin and Hb level ($r=0.463$, $p<0.001$) suggests that higher iron stores are associated with higher hemoglobin levels, highlighting the importance of adequate iron stores for preventing anemia. Negative correlation between ferritin and TIBC ($r=-0.189$, $p=0.026$) and a trend towards a negative correlation with UIBC ($r=-0.149$,

p=0.081) suggest that higher iron stores are associated with lower iron-binding capacities, as expected(Johnson Wimbley & Graham, 2011).

The median serum ferritin level was 14.239 ng/mL with an interquartile range of 6.968-24.571 ng/mL. About 34.1% of participants had serum ferritin levels below 10 ng/mL, indicating depleted iron stores, whereas 65.9% had normal levels. Ferritin is a marker of iron storage, and these findings suggest that while many women have adequate stores, a significant minority have depleted levels, which is concerning for their long-term health(Garcia-Casal et al., 2021). Increase in serum ferritin level with age concords with the previously conducted study on geriatric group which revealed that ferritin levels can rise with age(Cankurtaran et al., 2012). Ferritin, an iron storage protein, and transferrin receptors, which regulate the entry of iron-containing transferrin into cells, are important iron indicators. Serum ferritin levels correlate with tissue concentrations, with higher levels indicating more storage iron availability(Gebreegziabher & Stoecker, 2017). Higher intracellular iron concentrations promote ferritin expression, while iron deficiency inhibits it. However, Ferritin, an acute-phase protein, is found in higher concentrations during inflammation , even when there is iron deficiency, inflammatory processes can lead to elevated ferritin levels (Daru et al., 2017). The current study portrayed similar data with iron decreasing, and ferritin increasing with age.

The overall average serum iron level was 178.529±65.764 μg/mL, which is above the normal range (37-145 μg/mL). Interestingly, while 37.7% had normal serum iron levels, a considerable 62.3% had higher-than-normal levels. Elevated serum iron could be a result of various factors, including supplementation, diet, or less commonly, iron metabolism disorders.

Iron status disparities among ethnic groups have been reported and are still a topic of interest in the United States. An NHANES analysis found that African American men have lower haemoglobin and higher serum ferritin (SF) concentrations than non-Hispanic whites, as do African American post-menopausal women. However, no similar analysis is available for women of reproductive age or pregnant(Gordeuk & Brannon, 2017). As per the World Health Organisation, the acknowledged limit for anaemia in non-pregnant females over 15 years old living at sea level is 120 g/L. The average hemoglobin level was 12.091±1.159 g/dL. Within this cohort, 37.7% had low Hb levels, indicative of anemia, while 62.3% had Hb levels within the normal range. This prevalence of anemia is noteworthy and suggests a significant public health concern that requires addressing through nutritional and medical interventions(Let et al., 2024).

Haemoglobin levels are one of the indicators of iron status and fluctuate according to age, gender, pregnancy status, altitude, and smoking. Hb concentration has been used in some

studies to indicate IDA (Gebreegziabher & Stoecker, 2017). However, A new study suggests that the World Health Organization's diagnostic criteria for anaemia, based on a healthy Western population, may overestimate the frequency of the condition in children and women of reproductive age in India("Anaemia in Indian Women May Be Overestimated," 2023). Anaemia is diagnosed when a person's haemoglobin concentration is below the usual level for their age, gender, and physiological status. Adding measures of iron-deficient erythropoiesis, such as transferrin iron saturation, increases sensitivity(Gebreegziabher & Stoecker, 2017). Women of reproductive age exhibit higher iron storage and ID, influenced by ethnicity.(Gordeuk & Brannon, 2017) Female individuals with higher SF concentrations often had only mild to moderate increases in body iron. The therapeutic significance of such increases in iron reserves is questionable. Elevated body iron storage can lead to symptoms of porphyria cutanea tarda(Ryan Caballes et al., 2012). Elevated levels may increase the risk of cancer, including hepatocellular carcinoma without cirrhosis, diabetes mellitus, chronic hepatitis C infection, and non-alcoholic steatohepatitis(Faruqi et al., 2024; Ginzburg & Shaz, 2013). Future research should investigate the benefits and hazards of marginally or moderately high iron reserves during pregnancy, as they are currently unknown. The study found that while iron reserves are less common in women of reproductive age than ID, they are more prevalent in certain subgroups, including HFE C282Y homozygotes and African Americans.(Gordeuk & Brannon, 2017)

The study's findings indicate significant variability in iron status among women of reproductive age, with a notable prevalence of anaemia and iron deficiency. The correlations between age, iron parameters, and other biochemical markers provide insights into how iron metabolism might change with age and its interplay with other physiological processes. Given the high percentage of participants with abnormal iron levels, there is a clear need for targeted interventions. Furthermore, the associations observed between iron status and other health indicators like TSH and glucose suggest that comprehensive health assessments should consider multiple parameters to better understand and address the health needs of women in this age group.

CONCLUSION

The study indicates significant variability in iron status among women of reproductive age, with a notable prevalence of anemia and iron deficiency. Furthermore, the associations observed between iron status and other health indicators like TSH and glucose suggest that

comprehensive health assessments should consider multiple parameters to better understand and address the health needs of women in this age group.

Limitation of the study:

The study has relatively small sample size and lack of longitudinal data, it nonetheless highlights the need for comprehensive iron profiling in women of reproductive age.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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