Iron status in healthy elderly people: an evaluation of the role of soluble transferrin receptors in elderly

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Objective

The aim of this study was to examine the status of iron and the significance of soluble transferrin receptors (sTfR) in healthy elderly population.

Participants and methods

This study was carried out on 30 healthy elderly individuals (15 men and 15 women) above 65 years of age (the elderly group); in addition, 10 young participants served as controls (the control group). Serum iron level, total capacity of iron binding (TIBC), ferritin, and hemoglobin were measured. The level of sTfR was measured with a commercial kit using BioVendor Humans sTfR ELISA.

Results

Significant statistical decrease in hemoglobin level (P = 0.0007), ferritin (P = 0.00001), and serum iron (P = 0.00001), and a significant statistical increase in sTfR (P = 0.0013) were found in the elderly group. There was no statistical significant difference in TIBC (P = 0.4719) and significant negative correlation between sTfR and ferritin, TIBC, hemoglobin, and serum iron.

Conclusion

Serum iron decreases with advancing age and sTfR level increases. sTfR is negatively correlated with serum iron level and can be used as a reliable marker for iron stores, as sTfR is not affected by acute inflammatory conditions. sTfR measurement is useful in the diagnosis of iron deficiency.

Keywords:
elderly, ferritin, serum iron, soluble transferrin receptors

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Introduction

Iron (Fe) is an essential metal ion for humans; although it is the fourth most abundant mineral in the earth's crust, its deficiency is the most prevalent nutritional deficiency worldwide [1]. It participates in a variety of vital physiological processes such as oxygen transportation, energy production in the brain by cytochrome oxidase, and as enzymatic cofactor in the synthesis of neurotransmitters and myelin [2,3]. The main consequence of iron deficiency is anemia, which allows us to estimate its prevalence in a given population indirectly by counting the red blood cells.

There is no epidemiological data available on iron deficiency in the elderly, but they are expected to have a higher prevalence of anemia than do the general population, as longevity is associated with a variety of physiological dysfunctions, chronic and inflammatory diseases, and occasionally inadequate diet that lowers reserves and the availability of Fe. Clinical manifestations of anemia in the elderly add to changes in sensory organs, increasing the risk of falls, with a decline in mobility [4].

When facing a patient with iron deficiency anemia (IDA), the hematimetric and ferrokinetic classical standards can be altered by concomitant anemia of chronic disease (ACD) secondary to infectious, neoplastic, or inflammatory diseases [5–7]. ACD is a consequence of the production of proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-α) and some anti-inflammatory cytokines (interleukin-10), which induce the reticuloendothelial system to store Fe, limiting its availability for erythropoiesis, decreasing the half-life of erythrocytes, inhibiting the production of erythropoietin, and decreasing the sensitivity of erythroid precursors to erythropoietin [8,9]. Thus, ACD by itself results in hypoferremia and hyperferritinemia, thereby complicating etiological diagnosis of patients with simultaneous IDA. Moreover, normal physiological levels of serum iron are difficult to establish in a population because of its circadian rhythm [10].

In Fe deficiency, the decreased serum iron concentration leads to an increase in total capacity of iron binding

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(TIBC) and a decreased saturation of the iron transporter transferrin. Ferritin and transferrin have the disadvantage of being acute phase reactants with limited value in the differential diagnosis of ACD from IDA [11]. The above considerations justify efforts to design a highly sensitive and specific test to detect iron deficiency, ideally before the development of anemia [12,13]. Staining of the iron deposits in the bone marrow remains the gold standard, but it is an invasive technique. Thus, we assessed the use of soluble transferrin receptor (sTfR) in the present study [14]. The sTfR is a transmembrane protein with two identical polypeptide chains. Iron delivery to erythroblasts is mediated by the interaction of plasma transferrin with cell surface transferrin receptors [15,16]. From the cell membrane the serum transferrin receptors (TfR)–transferrin–iron complex is internalized through an endocytic vesicle, and in the intracellular compartment iron dissociates from TfR–transferrin complex [17]. The iron remains in the cytosol, while the TfR–transferrin complex is recycled back to the cell surface. Virtually all cells have transferrin receptors on their surface, but in a normal adult, about 80% of them are in the erythroid marrow. sTfR present in human plasma are a truncated form of tissue receptor and exist as a transferrin-receptor complex [18,19]. The number of TfRs on the cell surface reflects the iron requirement, and iron deprivation has been shown to result in the prompt induction of transferrin receptor synthesis [20]. The aim of this study was to examine the iron status and the significance of sTfR in normal elderly population.

Participants and methods

This study was conducted from March 2014 to May 2015 on a total of 30 normal elderly individuals (15 men and 15 women) above 65 years of age; in addition, 10 young participants served as controls. This study was conducted in the Department of Internal Medicine, Alexandria University Hospital, Egypt; after being approved by the local Research Ethics Committee, an informed consent was obtained from all participants. The age of elderly participants was 66–83 years (mean = 72.84 ± 4.61) and that of the controls was 31–50 years (mean = 38.85 ± 7.09). Only healthy elderly individuals were selected for the present study and any participant suffering from any diseases was excluded. The exclusion criteria included chronic renal disease, chronic liver disease, thyroid disease, bleeding disorders, chronic inflammatory diseases, and participants receiving iron-replacement therapy. All participants were subjected to following after obtaining informed consent:

(1) Thorough history-taking.
(2) Complete physical examination.
(3) Routine laboratory investigations including the following:
    (a) Complete blood count, random blood sugar, lipid profile (serum cholesterol and triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), liver function tests [serum glutamic oxalacitic transaminase (SGOT) and serum glutamic pyrovic transaminase (SGPT), serum albumin, prothrombin activity, and time], and renal function tests (blood urea, serum creatinine).
(4) Specific laboratory investigation including the following:
    (a) Serum iron level.
    (b) Ferritin level.
    (c) TIBC.
    (d) sTfR.

Blood counts were measured using an automated analyzer. Serum ferritin was measured using an automated time-resolved immunofluorometric assay. Serum iron was measured using an iron FZ assay (Hoffman-LaRoche, Basel, Switzerland) based on a guanidine hydrochloride/ferrozine reaction. Serum transferrin receptor assays were carried out with a commercial kit using BioVendor Humans sTfR ELISA. Quality controls and samples were incubated in microplate wells precoated with monoclonal anti-human sTfR antibody. After 60 min of incubation and washing, monoclonal anti-human sTfR antibody, conjugated with horseradish peroxidase, was added to the wells and incubated for 60 min with captured sTfR. Following another round of washing, the remaining horseradish peroxidase conjugate was allowed to react with the substrate solution (TMB). The reaction was stopped by adding acidic solution, and the absorbance of the resulting yellow product was measured. The absorbance was proportional to the concentration of sTfR. A standard curve was plotted (absorbance values against concentrations of standards); the concentrations of unknown samples were determined using this standard curve [21].

Statistical analysis

Data were collected and fed into the personal computer. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS/version 20, Alexandria, Egypt) software.

Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

Results

Table 1 shows comparison between elderly participants and controls regarding different studied variables. There was a significant difference between the studied groups as regards hemoglobin level (Hb); it was higher in the control group than in the elderly group (P = 0.0007). There was no statistical significant difference between the two studied groups regarding TIBC (P = 0.4719). In addition, there was a statistical significant difference between the studied groups as regards serum ferritin level; the levels in the elderly group were lower than in the control group (P = 0.00001). Serum iron was significantly lower in the elderly group than in the control group (P = 0.00001), as shown in Fig. 1. In addition, sTfR were significantly higher in the elderly group than in the control group (P = 0.0013), as shown in Fig. 2, and the elderly group was significantly older than the younger group (P = 0.00001).

Table 1 Comparison between the cases and control regarding different studied variables

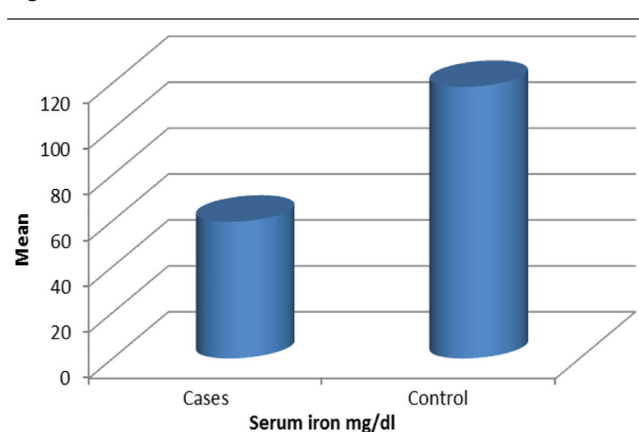
Item	Cases	Control	P
Hb (g/dl)			
Range	9.2–14.5	12–13.8	0.0007*
Mean	11.81	12.79	
SD	1.59	0.51	
TIBC (μg/dl)			
Range	251–409	263–400	0.4719
Mean	334.37	338.50	
SD	55.41	38.04	
Ferritin (ng/ml)			
Range	7–23	12–25	0.00001*
Mean	13.12	17.70	
SD	4.71	3.93	
Serum iron (mg/dl)			
Range	40–80	72–150	0.00001*
Mean	59.55	118.30	
SD	10.60	27.18	
STR (mg/dl)			
Range	2.0–3.8	2.0–2.5	0.0013*
Mean	2.68	2.27	
SD	0.56	0.18	
Age			
Range	66–83	31–50	0.00001*
Mean	72.84	38.85	
SD	4.61	7.09	

TIBC, total capacity of iron binding; *Statistical significant.

Table 2 shows the correlation between different studied variables in the elderly group. There was a negative correlation between TIBC and Hb (r = −0.840, P = 0.000). There was a positive correlation between serum ferritin level and Hb (r = 0.775, P = 0.000); however, there was a negative correlation between serum ferritin level and TIBC (r = −0.788, P = 0.000). As regards serum iron level, there was a positive correlation with Hb (r = 0.831, P = 0.000) and with ferritin (r = 0.667, P = 0.000), but a negative correlation with TIBC (r = −0.663, P = 0.000). In addition, there was a negative correlation between sTfR and Hb (r = −0.777, P = 0.000), ferritin (r = −0.692, P = 0.000), and serum iron (r = −0.624, P = 0.000); however, sTfRs were positively correlated with TIBC (r = 0.796, P = 0.000).

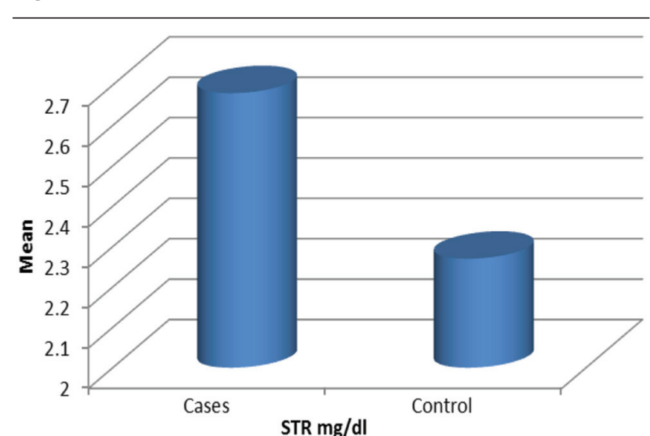
Table 3 shows comparison between men and women for different studied variables in the two groups. The hemoglobin level was significantly higher for elderly men than in elderly women participants (P = 0.041). There was no significant difference between men and women participants as regards TIBC (P = 0.113). Both ferritin and serum iron were significantly higher in men than in women participants (P = 0.045 and 0.032, respectively), as shown in Fig. 3. There was no significant difference between the two sexes as regards soluble transferrin receptors (STR) (P = 0.068), as shown in Fig. 4. There was no significant difference

Figure 1



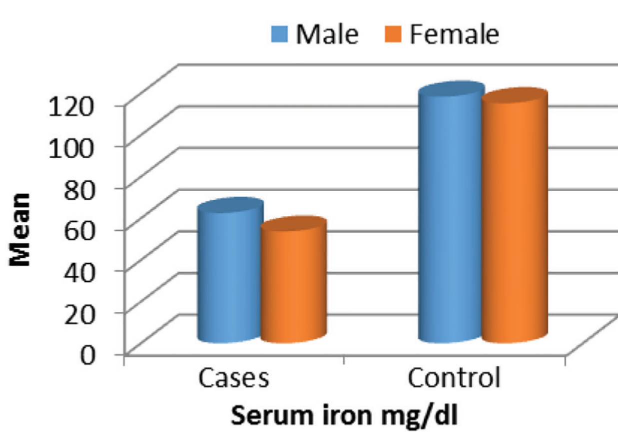
Comparison between the two studied groups.

Figure 2



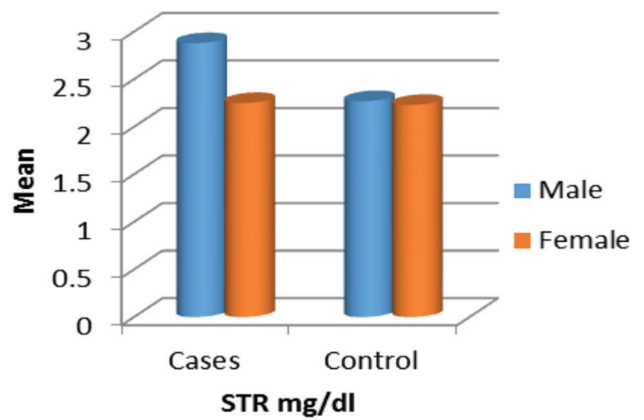
Comparison between the two studied groups according to serum iron according to STR.

Figure 3



Comparison between the elderly men participants.

Figure 4



Comparison between both sexes of the two elderly females according to serum iron studied groups according to STR.

Table 2 Correlation between different studied variables in cases group

Item	Hb (g/dl)	TIBC (μg/dl)	Ferritin (ng/ml)	Serum iron (mg/dl)
TIBC (μg/dl)				
r	–0.840**			
P	0.000			
Ferritin (ng/ml)				
r	0.775**	–0.788**		
P	0.000	0.000		
Serum iron (mg/dl)				
r	0.831**	–0.663**	0.667**	
P	0.000	0.000	0.000	
STR (mg/dl)				
r	–0.777**	0.796**	–0.692**	–0.624**
P	0.000	0.000	0.000	0.000

TIBC, total capacity of iron binding; **Statistical significant.

between men and women participants of the control group as regards all variables.

Discussion

In the current study, hemoglobin level was higher in the control group than in the elderly group, and it was higher in men than in women participants. This is in accordance with the changes that take place with aging; hemoglobin concentration has been reported to decline with advancing age, even in the absence of demonstrable disorders. In one study this was calculated to be 0.53 g/l/year in men and 0.05 g/l/year in women between the ages of 70 and 88 [22], and in another study the decline was 0.1 g/l/year in men and 0.09 g/l/year in women between the ages of 70 and 80 [23]. The decline appears to increase after the age of 80, particularly in men. It has been shown that growth hormone and/or insulin-like growth factor-1 is positively and erythropoietin is negatively correlated with hemoglobin in elderly people [24]. Erythrocytes released from the bone marrow function less and are partially damaged in aged individuals, and as these are not sufficiently able to protect themselves against stress, this results in their early sequestration [25]. We found no significant difference between the studied groups regarding TIBC; however, serum ferritin was significantly lower in the elderly group than in the young control group (P = 0.4719); this is in agreement with the findings of a study conducted by Hallberg *et al.* (1993) [26]. The fact that ferritin is a known acute-phase reactant also complicates its use as a marker of iron deficiency in acute or chronic inflammatory diseases. Serum/plasma ferritin concentration correlates closely with body iron stores, and values less than 12 μg/l indicate absence of liver iron stores. However, it is an acute phase protein and is elevated in people with infection or inflammation [27]. In the current study, serum ferritin was higher in the elderly men than in elderly women participants (P = 0.045); this in contrast to the results of a study conducted by Garry and colleagues (2000), who found that serum ferritin concentration was reported to be positively associated with increasing age in women (P = 0.0223) but not in men. However, in a longitudinal study undertaken in a subset of 125 people, there was no significant change in iron stores over the 10 years of monitoring period, suggesting that changes in serum ferritin (and iron stores) are not an inevitable consequence of aging [28]. Serum iron level was lower in elderly participants than in controls (P = 0.00001), and it was lower in elderly women than in men participants (P = 0.032). Chronic inflammation, a common condition in elderly people, alters iron metabolism, and hematopoiesis and can lead to anemia, but it is difficult to determine whether or not the cause of anemia is secondary to insufficient

Table 3 Comparison between male and female in different studied variables in all studied cases

Item	Cases		P	Control		P
	Male	Female		Male	Female	
Hb (g/dl)						
Range	10.8–14.5	9.2–13.5	0.041*	12.4–13.8	12.0–13.1	0.465
Mean	12.11	11.36		12.98	12.36	
SD	1.61	1.58		0.52	0.54	
TIBC (μg/dl)						
Range	271–409	251–382	0.113	271–400	263–389	0.152
Mean	336.88	326.2		346.50	339.2	
SD	55.98	49.5		38.41	42.6	
Ferritin (ng/ml)						
Range	8–23	7–20	0.045*	14–25	12–24	0.254
Mean	15.12	12.2		18.70	17.98	
SD	4.71	5.1		3.93	4.12	
Serum iron (mg/dl)						
Range	44–80	40–75	0.032*	74–150	72–142	0.125
Mean	62.5	53.6		118.30	115.2	
SD	11.2	11.5		27.18	26.8	
STR (mg/dl)						
Range	2.3–3.8	2.0–3.2	0.068	2.0–2.5	2.0–2.4	0.225
Mean	2.88	2.25		2.27	2.23	
SD	0.61	0.49		0.18	0.17	

TIBC, total capacity of iron binding.

iron supply because indices of iron status (notably serum iron, ferritin, and transferrin) are modified by the inflammatory state. It has been observed that malnutrition, not uncommon in the elderly, can exacerbate the effect of inflammation on biomarkers of iron status [29]. The bone marrow iron is considered as the accurate measure of the iron stores in the body, but it is an invasive technique, and thus we used sTfR as a marker for the evaluation of iron status.

It is important to remember that for most clinicians iron deficiency is associated with anemia; the truth is that it is a continuous process evolving in three stages. The first phase is the depletion of storage iron (stage I), where total body iron is decreased but hemoglobin (Hb) synthesis and red cell indices remain unaffected. Both these indexes change when the supply of iron to the bone marrow becomes problematic (iron-deficient erythropoiesis, or stage II). In stage III, the iron supply is insufficient to maintain a normal Hb concentration, and eventually IDA develops [30]. sTfR can detect stages II and III of iron deficiency. The sTfR is regarded as a more stable marker of iron levels in an inflammatory state.

In the current study, sTfR levels were higher in the elderly group than in the control group ($P = 0.0013$). This finding is in agreement with that of a study conducted by Flowers and colleagues (1989). The main source of serum sTfR is bone marrow erythroid precursors. When intracellular iron supply is reduced, cell surface transferrin 1 expression is upregulated to acquire more iron, and it is downregulated when there is sufficient iron. An elevated sTfR is a marker of tissue iron deficiency and increased bone marrow erythropoietic activity. The sTfR concentration increases in parallel with the severity of iron depletion [31]. The data from sTfR measurements give a clearer picture of functional iron status than do ferritin measurements. sTfR concentration remains unaffected by any active acute-phase reactants, which has made sTfR measurement an attractive tool for the differential diagnosis of IDA [32]. Its day-to-day variation is more acceptable than that of the conventionally used indices of functional iron status, such as transferrin saturation [33]. Humans use 80% of their body iron for erythropoiesis, and almost the same proportion of sTfRs in the body is found in the erythroid progenitor cells. Reticulocytes entering the peripheral bloodstream carry a high surface concentration of the receptor; as the cells mature the receptors are shed into the circulation [34]. Release of sTfRs has been shown to be mediated by an integral membrane proteinase, and inhibited by a matrix metalloproteinase and the TNF-N protease inhibitor-2 [35]. There was a negative correlation between sTfR and serum iron in our study.

Conclusion

Serum iron decreases with advancing age, even in the absence of anemia. sTfR is negatively correlated with serum iron level and can be used as a reliable marker for iron stores as sTfRs are not affected by the

acute inflammatory conditions. sTfR measurements are useful in the diagnosis of iron deficiency. It may be predicted that these measurements are likely to replace the conventional parameters of iron status – that is, serum iron, transferrin, and ferritin alone – in clinical laboratories. They would be especially useful at outpatient clinics, where bone marrow examinations are often either not available or are regarded as invasive means of identifying patients with depleted iron stores.

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Conflicts of interest
There are no conflicts of interest.

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