High Serum Iron Is Associated with Increased Cancer Risk

Chi Pang Wen[1,2], June Han Lee[1], Ya-Ping Tai[1], Christopher Wen[3], Shiuan Be Wu[1], Min Kuang Tsai[1], Dennis P.H. Hsieh[1,4], Hung-Che Chiang[5], Chao Agnes Hsiung[1], Chung Y. Hsu[6], and Xifeng Wu[7]

Abstract

Epidemiologic studies linking high serum iron with cancer risks are limited and inconclusive, despite evidence implicating body iron in human carcinogenesis. A cohort of 309,443 adults in Taiwan who had no history of cancer had serum iron levels tested at the time of recruitment (1997–2008). Initially measured iron levels were associated with subsequent cancer risk by linking individuals with the National Cancer Registry and National Death File. HRs were calculated by the Cox model. One third of males (35%) and one fifth of females (18%) had high serum iron ($\geq$120 μg/dL), which was associated with a 25% increase in risk for incidence of all cancers [HR, 1.25; 95% confidence interval (CI), 1.16–1.35] and with a 39% increase in risk for mortality from all cancers (HR, 1.39; 95% CI, 1.23–1.57). The relationship between serum iron and cancer risk was a J-shaped one, with higher cancer risk at both ends, either at lower than 60 μg/dL or higher than 120 μg/dL. At the higher end, cancer risk increased by 4% for every 10 μg/dL increment above 80 μg/dL, showing a dose–response relationship, with 60 to 79 μg/dL as a reference level. In a sensitivity analysis, the increases in risk were still observed after the first 5 years of cancer cases were excluded. Liver cancer risk was increased in HBV (−) non-hepatitis B carrier (3-fold) and HBV (+) hepatitis B carrier (24-fold). Lifestyle risks such as smoking, drinking, or inactivity interacted synergistically with high serum iron and significantly increased the cancer risks. The liver (HR, 2.49; 95% CI, 1.97–3.16) and the breast (HR, 1.31; 95% CI, 1.01–1.70) were the two major cancer sites where significant cancer risks were observed for serum iron either $\geq$120 μg/dL or $\geq$140 μg/dL, respectively. This study reveals that high serum iron is both a common disorder and a marker of increased risk for several cancers. *Cancer Res; 74(22); 6589–97. ©2014 AACR.*

Introduction

Although interest in linking high body iron with cancer risk in humans has spanned several decades, results from epidemiologic studies have been inconclusive. The most recent study from Sweden, with a sample size of more than 200,000, reported no association between serum iron and cancer risks at most sites (1). In earlier studies, both negative (2–5) and positive associations (6–8) have been described. Even among those studies reporting a positive correlation, the observed risk varied by cancer type and by gender (6–8). A recent clinical trial conducted at VA hospitals in the United States was a milestone study, in which a reduction of serum iron levels attained through phlebotomy was positively correlated with decreased cancer risk (9). The overall trend and the positive result reported were encouraging, but the study suffered from limited statistical power. The trial could only be considered a pilot because the expected iron–cancer dose–response relationship was not observed and consistent cancer types were not reported.

Though epidemiologic studies have yielded mixed results, there is a well-known iron–cancer relationship among patients with hemochromatosis, who are prone to develop liver cancer (10–12). As the basic mechanism related to iron-induced carcinogenesis seems well established (13–17), more definitive epidemiologic studies are needed. Using the serum iron data available from a large cohort of more than 300,000 adults in Taiwan, we prospectively assessed whether high serum iron was associated with increases in cancer occurrences or in cancer-related deaths among initially cancer-free subjects.

Patients and Methods

Study population and data collection

The study cohort consisted of 309,443 adults (145,088 men and 164,355 women) who were at least 20 years of age and had no history of cancer at the time of recruitment. The subjects participated in a standardized medical screening program between 1997 and 2008, with a median follow-up period of 7.07 years. The self-paying screening program was run by a private firm (MJ Health Management Institution, Taiwan) that attracted paying participants from all over Taiwan because of

[1]Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan. [2]China Medical University Hospital, Taichung, Taiwan. [3]Long Beach Veterans Administration Hospital, University of Irvine, California. [4]Department of Environmental Toxicology, University of California, Davis. [5]National Environmental Health Center, National Health Research Institutes, Zhunan, Taiwan. [6]Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan. [7]Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Corresponding Author: Chi Pang Wen, National Health Research Institutes, No. 35, Keyan Road, Zhunan, Miaoli, 350 Taiwan. Phone: 886-37-246-166 ext. 36318; Fax: 886-37-586-261; E-mail: cwengood@nhri.org.tw

doi: 10.1158/0008-5472.CAN-14-0360

its known quality services, operational efficiency, and accessible key facilities. The majority of cohort members would come back for repeated examinations in subsequent years, but only test results from the initial examination were used in our analysis. A detailed description of the cohort has been documented elsewhere (18).

Each subject completed a self-administered questionnaire with medical history, lifestyle, and demographic information. Regarding smoking status, individuals were classified as nonsmokers, current smokers, or ex-smokers. Regular drinkers were those consuming two or more alcoholic beverages at least three times per week. Leisure time physical activity (LTPA) volume, the product of intensity (metabolic equivalent, MET) and duration of exercise (hours) were classified into three levels: inactive (<3.75 MET h/wk), somewhat active (15–29 min/d or 3.75–7.49 MET h/wk), and fully active (≥30 min/d or ≥7.5 MET h/wk), with the fully active category comprised of individuals who met the current LTPA recommendation (19). Anemia was defined as a hemoglobin level below 13 g/dL for men and below 12 g/dL for women. HBV (+) subjects were individuals with a positive hepatitis B surface antigen test and HCV (+) subjects were individuals with a positive hepatitis C antibody test.

Serum iron was measured by a Nitroso-PSAP method, which used acid to dissociate the Fe(III)–transferrin complex, and Fe (II) was then assessed with an Abbott Architect C8000 automatic biochemistry analyzer.

Informed consent was obtained to authorize data processing and analysis. Ethical reviews were approved by the Institutional Review Boards at the National Health Research Institutes (Taiwan). Individually identifying data were removed and remained anonymous during the entire study process.

Follow-up

Using the unique national identification numbers, subjects were each matched with the National Cancer Registry and National Death File between 1997 and 2008. A total of 8,060 cancer incidents and 3,066 cancer-related deaths were identified.

Statistical analysis

Cox proportional hazard analysis was used to calculate HRs. The HRs were adjusted for 10 confounding variables, including six continuous and four categorical variables. The continuous confounding variables were age, BMI, systolic blood pressure, total cholesterol level, C-reactive protein level, and hemoglobin level; the categorical variables were gender, smoking (nonsmoker, ex-smoker, and current smoker), drinking (never or occasional drinker, regular drinker), physical activity (inactive, somewhat active, and fully active), HBV (hepatitis B surface antigen carrier status), and HCV (hepatitis C antibody carrier status). Attributable fraction (AF) estimates the proportion of cancer cases, which could be avoided if high serum iron were eliminated. $AF = (R_1–R_0)/R_1 = (RR–1)/RR$, where R_1 is the cancer incidence among high serum iron, R_0 is the cancer incidence among of the reference group, and RR (relative risk) $= R_1/R_0$. In this study, AF was estimated as (HR-1)/HR. Relative excess risk due to interaction (RERI) was used to determine whether the combined effect of two exposures, high serum iron and each risk factor, was larger than the sum of the single effects from each exposure as a reflection of synergistic interaction. The value of RERI > 0 indicates a positive synergy, RERI = 0, no synergy, and RERI < 0 antagonistic interaction (20).

All statistical tests were two-sided, with the alpha level set at 0.05. Analyses were performed with SAS, version 9.2.

Results

One quarter of the cohort (26.2%) had high serum iron, defined as ≥120 μg/dL (Table 1). Serum iron levels were consistently higher in males than in females (means of 109 and 88 μg/dL, respectively), regardless of age. By adjusting age and education levels of the cohort to those of Taiwan, the national prevalence of high serum iron was estimated. It was found that nationally, twice as many males as females had high serum iron (35% and 19%, respectively; Fig. 1), a figure similar to that observed in this cohort.

We observed a dose-dependent relationship between high serum iron and cancer, with a 4% increase in all cancer incidence risk for each 10 μg/dL increment of serum iron above 80 μg/dL (Fig. 2 and Supplementary Fig. S1). On the other hand, a serum iron level below 60 μg/dL also conferred a significantly increased cancer risk [HR, 1.18; 95% confidence interval (CI), 1.08–1.29]. As a result, when using 60 to 79 μg/dL as a reference level, the plot of cancer risk against serum iron revealed a J-shaped relationship with two major increases in risk observed, one above 80 μg/dL and one below 60 μg/dL.

Subjects with high serum iron (≥120 μg/dL) had a 25% increase in cancer incidence risk (HR, 1.25; 95% CI, 1.16–1.35; Table 2) and a 39% increase in cancer-related mortality risk (HR, 1.39; 95% CI, 1.23–1.57; Supplementary Table S2). There was a 2.49-fold increase in liver cancer incidence (HR: 2.49, 95% CI: 1.97–3.16) for males and females combined who had high serum iron and a 31% increase in breast cancer incidence (HR, 1.31; 95% CI, 1.01–1.70) for females with serum iron ≥140 μg/dL. Although the results were adjusted for HBV and HCV, which are the two major risk factors for liver cancer, additional analyses were conducted on sub-cohorts stratified by HBV status, with all HCV (+) subjects excluded. With HBV (–) and HCV (–) subjects shown in Table 3, their liver cancer risk was increased by approximately 3-fold (HR, 3.19; 95% CI, 2.23–4.57). Compared with the HBV (–) population with a serum iron level of 60 to 79 μg/dL, cancer risks were increased among subjects either with high serum iron alone (HR, 1.14 for all cancer and 3.19 for liver cancer) or with HBV alone (HR, 1.33 for all cancer and 12.14 for liver cancer). However, a synergistic increase was found when both risks were present (HR, 2.12 for all cancer and 24.38 for liver cancer; Table 3).

A forest plot of the data in Fig. 3 shows that the increase in cancer risk was significant regardless of gender, smoking status, drinking, physical activity, or HBV infection. In addition, the increased risk was still observed when the first 5 years of cancer cases were excluded (Fig. 3).

Fig. 4 shows the cancer risk of smoking, drinking, and inactivity individually and in combination with high serum iron in a multivariate analysis. The effect of smoking, drinking,

Table 1. Characteristics of the cohort by serum iron levels

Serum iron (μg/dL)	All	<60 *N* (%)	60–79 *N* (%)	80–119 *N* (%)	≥120 *N* (%)	≥140 *N* (%)
Total	309,443	46,090 (14.9)	56,111 (18.1)	126,240 (40.8)	81,002 (26.2)	41,950 (13.6)
Age, mean (SD)	41.8 (14.0)	40.3 (13.5)	42.7 (14.5)	42.8 (14.2)	40.4 (13.4)	39.6 (13.1)
Gender						
Male	145,088	11,002 (7.6)	21,565 (14.9)	61,597 (42.5)	50,924 (35.1)	28,039 (19.3)
Female	164,355	35,088 (21.3)	34,546 (21.0)	64,643 (39.3)	30,078 (18.3)	13,911 (8.5)
Age						
20–39	160,872	25,818 (16.0)	28,006 (17.4)	61,989 (38.5)	45,059 (28.0)	24,346 (15.1)
40–64	125,022	17,121 (13.7)	22,995 (18.4)	53,602 (42.9)	31,304 (25.0)	15,532 (12.4)
≥65	23,549	3,151 (13.4)	5,110 (21.7)	10,649 (45.2)	4,639 (19.7)	2,072 (8.8)
Smoking status						
Nonsmoker	210,278	35,713 (17.0)	40,983 (19.5)	86,795 (41.3)	46,787 (22.3)	22,394 (10.6)
Ever smokers[a]	85,998	8,235 (9.6)	12,646 (14.7)	34,114 (39.7)	31,003 (36.1)	17,935 (20.9)
Drinking status						
Never or occasional	230,237	36,925 (16.0)	43,555 (18.9)	94,632 (41.1)	55,125 (23.9)	27,340 (11.9)
Regular drinker	59,480	5,817 (9.8)	8,733 (14.7)	23,597 (39.7)	21,333 (35.9)	12,340 (20.7)
Physical activity						
Inactive	168,133	27,378 (16.3)	30,632 (18.2)	66,832 (39.7)	43,291 (25.7)	22,797 (13.6)
Somewhat active	61,822	9,088 (14.7)	11,210 (18.1)	25,274 (40.9)	16,250 (26.3)	8,392 (13.6)
Fully active	75,842	9,110 (12.0)	13,628 (18.0)	32,521 (42.9)	20,583 (27.1)	10,317 (13.6)
BMI (kg/m^2)						
<18.5	26,291	5,105 (19.4)	4,746 (18.1)	9,768 (37.2)	6,672 (25.4)	3,531 (13.4)
18.5–24	198,954	30,810 (15.5)	36,169 (18.2)	80,076 (40.2)	51,899 (26.1)	26,990 (13.6)
25–29	71,924	8,505 (11.8)	12,769 (17.8)	31,174 (43.3)	19,476 (27.1)	9,930 (13.8)
≥30	12,274	1,670 (13.6)	2,427 (19.8)	5,222 (42.5)	2,955 (24.1)	1,499 (12.2)
Fasting glucose (mg/dL)						
<126	291,700	43,855 (15.0)	52,474 (18.0)	118,387 (40.6)	76,984 (26.4)	40,034 (13.7)
≥126 or diabetes[b]	17,743	2,235 (12.6)	3,637 (20.5)	7,853 (44.3)	4,018 (22.6)	1,916 (10.8)
Systolic blood pressure (mm Hg)						
<140	251,762	38,652 (15.4)	44,648 (17.7)	101,131 (40.2)	67,331 (26.7)	35,282 (14.0)
≥140 or hypertension[c]	57,681	7,438 (12.9)	11,463 (19.9)	25,109 (43.5)	13,671 (23.7)	6,668 (11.6)
Total cholesterol (mg/dL)						
<240	271,535	42,288 (15.6)	48,931 (18.0)	108,603 (40.0)	71,713 (26.4)	37,550 (13.8)
≥240[d]	37,908	3,802 (10.0)	7,180 (18.9)	17,637 (46.5)	9,289 (24.5)	4,400 (11.6)
C-reactive protein (mg/dL)						
<1	300,171	41,770 (13.9)	54,029 (18.0)	124,075 (41.3)	80,297 (26.8)	41,624 (13.9)
≥1	9,264	4,319 (46.6)	2,079 (22.4)	2,163 (23.3)	703 (7.6)	325 (3.5)
Anemia[e]						
No	285,858	34,217 (12.0)	52,325 (18.3)	120,606 (42.2)	78,710 (27.5)	40,871 (14.3)
Yes	23,585	11,873 (50.3)	3,786 (16.1)	5,634 (23.9)	2,292 (9.7)	1,079 (4.6)
Hepatitis B carrier status (HBV)						
Negative	261,621	39,847 (15.2)	48,284 (18.5)	107,236 (41.0)	66,254 (25.3)	33,829 (12.9)
Positive	44,232	5,609 (12.7)	7,134 (16.1)	17,624 (39.8)	13,865 (31.3)	7,696 (17.4)

[a]Ever smokers included current smokers and ex-smokers.

[b]Included are those on diabetes medication or with history of diabetes.

[c]Included are those on hypertension medication or with history of hypertension.

[d]Included are those on high-cholesterol medication.

[e]Anemia is defined as hemoglobin <13 g/dL for men and <12 g/dL for women.

or inactivity combined with high serum iron produced a roughly 50% extra-increase in the already-elevated cancer risks, from 1.13 to 1.74 for smoking (RERI, 0.27; 95% CI, 0.12–0.42), 1.14 to 1.61 for drinking (RERI, 0.24; 95% CI, 0.08–0.39), and 1.17 to 1.85 in combined risks (RERI, 0.36; 95% CI, 0.10–0.61). These interactions between these risks and high serum iron were synergistic and statistically significant (RERI > 0 and the 95% CI did not contain 0).

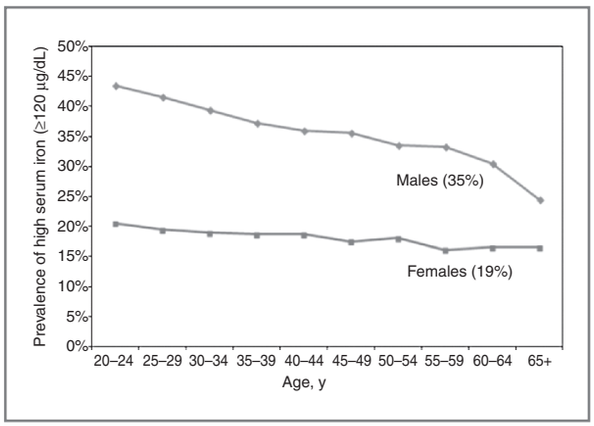

Figure 1. National prevalence of high serum iron (≥120 μg/dL) for Taiwan, projected from the study cohort, by adjusting educational levels to those of Taiwan. The national prevalence of high serum iron for males and females was estimated by adjusting the study population to that of the Taiwan 2012 data by age (in 5-year groupings) and three education levels (middle school or lower, high school or junior college, and college or higher). The detailed information on the adjustment method was reported elsewhere (18).

Given the increased HR of 1.25 for those with high serum iron, the AF from high serum iron was 20%, based on the formula AF = (HR-1)/HR.

Discussion

In this study, high serum iron is found to be a common disorder and a strong marker for cancer incidence or mortality. The relationship between serum iron and cancer risk was a J-shaped one, with higher cancer risk at both ends, at lower than 60 μg/dL and higher than 120 μg/dL (Fig. 2). At higher end, there was a dose–response relationship with cancer risk increased by 4% for every 10 μg/dL increment above 80 μg/dL with 60 to 79 μg/dL as a reference level. For serum iron above 120 μg/dL, a 25% increase in cancer incidence and 39% increase in cancer-related mortality was found. At even higher

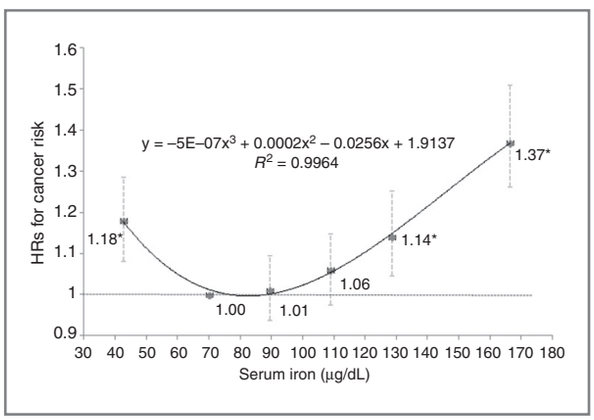

Figure 2. J-shaped relationship between serum iron levels and cancer risk. The reference group, serum iron level at 60 to 79 μg/dL. Those with serum iron at both ends, <60 and ≥120 μg/dL, had significant increase in cancer risk ($P < 0.05$; Table 2); *, $P < 0.05$.

serum iron levels (above 140 μg/dL), cancer risk increased by 37% (HR, 1.37; 95% CI, 1.26–1.50) for both genders combined and by 46% (HR, 1.46; 95% CI, 1.30–1.65) for males. This increase is a magnitude that is comparable with or higher than the increases in cancer risk associated with lifestyle factors such as tobacco smoking (HR, 1.67; ref. 21), obesity (HR, 1.14; ref. 22), or physical inactivity (HR, 1.20; ref. 19) in this study cohort.

Under the definition of high serum iron as ≥120 μg/dL, we found that as many as one third of males (35%) and one fifth of females (18%) fell into this classification. This is a large proportion of the general population with increased cancer risk. To assess whether this high prevalence is unique to Taiwan, we compared the numbers from Taiwan with those from the United States. The U.S. data came from the National Health and Nutrition Examination Survey (NHANES) 2001 to 2006 (23). The U.S. prevalence of high serum iron was 21% for males and 14% for females, which are figures that are substantially lower than those observed in Taiwan. Nevertheless, the proportion of individuals with high serum iron in the United States (1/5 males and 1/7 females) was still remarkable.

To our knowledge, this study is the first large population study to epidemiologically show the association of high serum iron with increased risk for all cancers combined and for liver cancer and breast cancer specifically. The same association was observed for selected nonliver cancers, which were defined as cases from all sites excluding cases of liver cancer, stomach cancer, colorectal cancer, and pancreatic cancer from all cancer cases. When these sites with excesses were combined, we found the overall excess risk to be statistically significant. It is evident that the positive association between serum iron levels and cancer risk was not limited to liver cancer. We also conducted a number of sensitivity analyses to strengthen our findings. We separated smokers from nonsmokers as well as HBV carriers from noncarriers, and we found that the increased cancer risk from high serum iron persisted in all groups (Table 3 and Supplementary Table S1). We also excluded cancer cases that occurred in the first 5 years and found that the association was still observed (Fig. 2). The fact that as many as 10 variables were controlled for in our Cox model lends further support to the independent effect of high serum iron on cancer risk. We also analyzed cancer-related mortality and found results similar to those for cancer incidence (Supplementary Table S2). Our results support the notion that body iron plays an important role in human carcinogenesis (14, 16, 17).

The role of iron overload in liver cancer development has been well documented for genetic hemochromatosis (10–12) and nonhemochromatosis (24) as well as for HBV (+) conditions (25, 26). However, we are the first to report a definite association between high serum iron and a large increase in risk of liver cancer in a population at average risk. Although hemochromatosis is associated with increased liver cancer risk, the prevalence of hemochromatosis in Taiwan is very low, approximately 0.3% to 0.5% (27, 28). This prevalence translates into 3 to 5 cases per thousand individuals among Caucasians, with estimates being lower for Asians (29, 30). Especially when compared with the 18% to 35% of subjects with high serum iron

Table 2. Cancer incidence risk for different serum iron levels by cancer types

		<60		60–79		80–99		100–119		≥120		≥140		
Serum iron (μg/dL)	***N***	***n***	**HR 95% CI**	***n***	**HR**	***n***	**HR 95% CI**	***n***	**HR 95% CI**	***n***	**HR 95% CI**	***n***	**HR 95% CI**	P_{trend}
Total														
All cancer	8,060	1,302	1.18 (1.08–1.29)[a]	1,469	1.00	1,752	1.01 (0.94–1.09)	1,456	1.06 (0.97–1.15)	2,081	1.25 (1.16–1.35)[a]	1,118	1.37 (1.26–1.50)[a]	<0.0001
Liver	989	122	1.42 (1.05–1.90)[a]	117	1.00	147	1.08 (0.82–1.42)	178	1.46 (1.12–1.90)[a]	425	2.49 (1.97–3.16)[a]	275	2.98 (2.32–3.84)[a]	<0.0001
Lung	856	126	1.15 (0.88–1.50)	163	1.00	207	1.14 (0.91–1.42)	159	1.08 (0.85–1.38)	201	1.05 (0.83–1.33)	102	1.08 (0.82–1.42)	
Nasopharynx	165	22	1.69 (0.85–3.37)	19	1.00	48	1.96 (1.10–3.49)[a]	30	1.26 (0.67–2.38)	46	1.43 (0.79–2.57)	20	1.09 (0.54–2.18)	
Esophagus	88	9	0.65 (0.23–1.86)	13	1.00	19	1.19 (0.54–2.66)	20	1.59 (0.73–3.47)	27	1.22 (0.57–2.58)	18	1.52 (0.69–3.38)	
Kidney	266	35	0.77 (0.46–1.31)	57	1.00	67	1.07 (0.72–1.61)	48	0.98 (0.63–1.52)	59	1.02 (0.66–1.56)	28	1.04 (0.62–1.75)	
Selected nonliver cancer[b]	4,978	787	1.09 (0.98–1.22)	971	1.00	1,123	0.99 (0.90–1.09)	922	1.03 (0.93–1.14)	1,175	1.11 (1.01–1.22)[a]	605	1.17 (1.04–1.32)[a]	0.0027
Male														
All cancer	4,177	507	1.39 (1.21–1.59)[a]	594	1.00	858	1.08 (0.96–1.21)	796	1.13 (1.01–1.28)[a]	1,422	1.36 (1.22–1.52)[a]	812	1.46 (1.30–1.65)[a]	<0.0001
Liver	688	70	2.17 (1.45–3.24)[a]	61	1.00	97	1.30 (0.90–1.89)	127	1.74 (1.22–2.49)[a]	333	2.78 (2.01–3.85)[a]	223	3.24 (2.31–4.53)[a]	<0.0001
Lung	516	65	1.36 (0.94–1.98)	75	1.00	127	1.31 (0.96–1.78)	93	1.11 (0.80–1.55)	156	1.14 (0.84–1.55)	88	1.21 (0.86–1.70)	
Colorectal	572	94	1.79 (1.29–2.48)[a]	83	1.00	134	1.00 (0.74–1.35)	102	0.94 (0.69–1.29)	159	0.97 (0.73–1.30)	74	0.89 (0.64–1.25)	
Prostate	497	52	0.99 (0.66–1.46)	94	1.00	119	0.91 (0.67–1.24)	103	0.93 (0.68–1.27)	129	0.82 (0.60–1.12)	63	0.79 (0.55–1.15)	
Female														
All cancer	3,883	795	1.03 (0.92–1.16)	875	1.00	894	0.97 (0.87–1.08)	660	1.00 (0.90–1.13)	659	1.15 (1.03–1.29)[a]	306	1.32 (1.14–1.52)[a]	0.0048
Liver	324	54	0.85 (0.54–1.32)	60	1.00	52	0.87 (0.57–1.32)	56	1.20 (0.79–1.82)	102	2.53 (1.75–3.66)[a]	52	3.39 (2.22–5.18)[a]	<0.0001
Lung	340	61	0.99 (0.68–1.44)	88	1.00	80	0.96 (0.68–1.34)	66	1.09 (0.76–1.55)	45	0.93 (0.62–1.38)	14	0.78 (0.43–1.42)	
Breast	913	166	0.81 (0.65–1.02)	208	1.00	204	0.83 (0.67–1.02)	159	0.85 (0.68–1.07)	176	1.10 (0.89–1.36)	88	1.31 (1.01–1.70)[a]	
Colorectal	454	102	1.20 (0.88–1.63)	105	1.00	126	1.12 (0.85–1.49)	72	0.92 (0.66–1.28)	49	0.64 (0.40–1.04)	19	0.93 (0.56–1.53)	

NOTE: HR was adjusted for age, gender, BMI, systolic blood pressure, total cholesterol, C-reactive protein, hemoglobin, smoking, drinking, and physical activity in a multivariate Cox model. HR for liver cancer was additionally adjusted for HBV among subjects without liver cirrhosis and HCV (+).

[a]A significantly ($P < 0.05$) higher incidence compared with the group with 60 to 79 μg/dL serum iron.

[b]Except for liver cancer, stomach cancer, colorectal cancer, pancreatic cancer, and thyroid cancer, all other cancer codes were categorized into "selected nonliver cancer."

Table 3. Cancer incidence risk in HBV (+) sub-cohort[a] and HBV (−) sub-cohort[a] by different serum iron levels

	<60			60–79			80–99			100–119			≥120			≥140	
Serum iron (μg/dL)	Total	Case	HR 95% CI	Case	HR 95% CI	case	HR 95% CI	Case	HR 95% CI	Case	HR 95% CI	Case	HR 95% CI	Case	HR 95% CI	Case	HR 95% CI
All cancer incidence																	
HBV (−)	6,467	1,098	1.17 (1.07–1.29)[b]	1,253	1.00 (reference)	1,456	1.00 (0.92–1.09)	1,164	1.04 (0.95–1.13)	1,496	1.14 (1.05–1.24)[b]	761	1.22 (1.10–1.35)[b]				
HBV (+)	1,423	184	1.73 (1.45–2.06)[b]	190	1.33 (1.12–1.58)[b]	274	1.51 (1.30–1.75)[b]	271	1.60 (1.38–1.85)[b]	504	2.12 (1.89–2.38)[b]	308	2.34 (2.04–2.70)[b]				
Liver cancer incidence																	
HBV (−)	449	61	1.55 (1.00–2.39)[b]	54	1.00 (reference)	77	1.45 (0.97–2.16)	82	1.87 (1.26–2.78)[b]	175	3.19 (2.23–4.57)[b]	104	3.98 (2.71–5.84)[b]				
HBV (+)	535	59	16.03 (10.48–24.51)[b]	63	12.14 (7.98–18.46)[b]	69	9.80 (6.46–14.86)[b]	95	14.22 (9.60–21.06)[b]	249	24.38 (17.14–34.67)[b]	162	28.49 (19.73–41.14)[b]				

NOTE: HR was adjusted for age, gender, BMI, systolic blood pressure, total cholesterol, C-reactive protein, hemoglobin, smoking, drinking, and physical activity continuous when appropriate. Trend tests are significant in two tests ($P < 0.0001$).

Abbreviations: HBV (+), positive for hepatitis B surface antigen test; HCV (+), positive for hepatitis C antibody test.

[a]Subjects with HCV (+) excluded.

[b]A significantly ($P < 0.05$) higher incidence compared with HBV (−) subjects with 60 to 79 μg/dL serum iron.

in this study, the prevalence of hemochromatosis is so low that its effect on the development of liver cancer could be ignored in this large prospectively followed cohort.

The association between high serum iron and elevated liver cancer risk remained regardless of HBV or HCV infection, but a strong synergistic interaction between high serum iron and HBV (+) existed when both were present. The increased risk for liver cancer (HR, 24.38; 95% CI, 17.14–34.67) conferred by high serum iron and HBV (+) acting together far exceeded the sum of the increased risks from high serum iron (HR, 3.19; 95% CI, 2.23–4.57) and HBV [(+) (HR, 12.14; 95% CI, 7.98–18.46)] acting alone (Table 3). Incidentally, the prevalence of HBV in this cohort was high—17% for males and 12% for females—though this is commonly observed in Asian countries, including Taiwan (31, 32). The significant increase in cancer risk associated with high serum iron in the non-HBV carrier cohort supports the idea that high serum iron plays a role in increasing the liver cancer and all cancer risks, which are independent of HBV status.

In our study, we found a significant 31% increase in breast cancer risk among women with serum iron levels higher than 140 μg/dL. This finding is consistent with the prevailing hypothesis that iron overload was known to increase breast cancer risk (33, 34). Epidemiologically, this finding of a breast cancer risk increase in women before or after menopause is new, as the existing studies have focused mainly on older age groups (1, 35). Because high serum iron is a risk factor for breast cancer, oral iron supplementation for women may be contraindicated. If our results are clinically confirmed, iron salts should be reconsidered as an ingredient in the vitamin pills regularly consumed by women without overt anemia.

The increased cancer risk associated with high serum iron was enhanced by three lifestyle risk factors: smoking, drinking, and inactivity (RERI, 0.36; 95% CI, 0.10–0.61). We used RERI to determine the presence of synergy between two or more risks. As each of these risks, high serum iron and each of the three life style risks, had the property of increasing cancer risk, one wonders to whether the combined effect was just a sum of the two or significantly larger than the sum. When found to be significantly larger, by the RERI test, the combination was said to have synergistic interaction. In this study, the presence of one of the three risk factors, along with high serum iron increased cancer risk over and above the sum of their individual risk increases (Fig. 4). A reasonable assumption from this result is that an effort to reduce some of these lifestyle factors could substantially reduce cancer risks. As there are limited options for reducing high serum iron, we believe that the pursuit of modifying life style risks should be considered for every patient. Of course, encouraging periodic blood donations may be another effective way to reduce cancer risk, as demonstrated in a pilot study (9).

We looked into the differences between the recent Swedish study (which found a null association) and our study, and we found that the selection of the reference group was critical. We observed an increased cancer risk (HR, 1.18; 95% CI, 1.08–1.29) among those with low serum iron. Colorectal or stomach cancer is known to be associated with iron deficiency anemia from chronic blood loss. As a result, the correlation of cancer

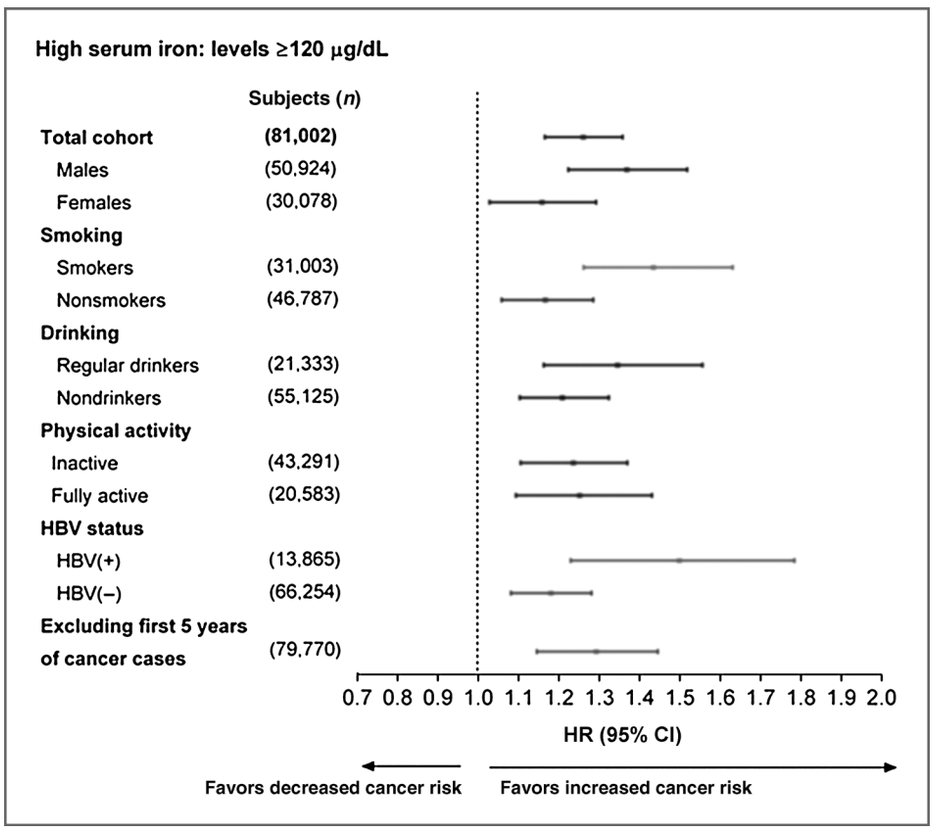

Figure 3. Forest plot showing increased cancer risk for different subgroups with high serum iron (≥120 μg/dL). The cohort was cancer free at entry. The reference group, serum iron level at 60 to 79 μg/dL. Regular drinkers, those consuming ≥2 alcoholic beverages ≥3 times per week. Fully active, those with ≥30 minutes/day of moderate intensity or ≥7.5 MET-hour. HBV (+), hepatitis B surface antigen positive, excluding first 5 years of cancer cases. Analysis made with the first 5 years of cancer cases excluded.

risk with serum iron level was not linear but J-shaped, with the lowest serum iron level (<60 μg/dL) having a significantly increased cancer risk. As such, the lowest quartile of serum iron level should not be used as a reference group, as doing so could mask the increased cancer risk among those with higher serum iron (Fig. 2). A rough calculation using the second quartile of the Swedish study as a reference instead of the first quartile found that the cancer risk ratio in the highest quartile of serum iron level would have changed from 0.96, indicating no association or a slightly decreased risk, to 1.06, indicating a slightly increased risk. We could not determine the significance of this new ratio without access to the original data. However, this observation is compatible with a J-shaped association in the Swedish study.

There are limitations to this study. First, we focused our analysis on serum iron, one of several biomarkers of iron status, which may or may not correlate with body iron stores (4, 36). A recent meta-analysis of 59 epidemiologic studies concluded that biomarkers of iron stores tend to be negatively correlated with cancer risk (37). It should be noted that our study did not aim to assess the correlation of cancer risk with body iron stores, but with serum iron. Without adjusting for body iron stores, we were able to establish a link and a dose–response relationship between serum iron levels and the risk for cancer incidence and mortality. As serum iron is a relatively inexpensive and widely available test, tests can be ordered and high serum iron could be found and interpreted in daily practice, making our results clinically relevant. A second study limitation was that the average follow-up time from when serum iron was tested was 7 years, which could be viewed as too short a time for cancer to develop. Questions may arise to whether there was a sufficient length of time for the elevated serum iron to affect cancer development, as the mean follow-up time was approximately 7 years, with the longest being 14 years. When the prevalence of high serum iron was examined across age groups, we observed high serum iron levels at all ages, with higher ones in earlier years. A third limitation is that the conclusions from this study may not be applicable to non-Asians because the cohort came from Taiwan. However, similar results were found from several studies on Caucasians (6, 7), whereas reductions of cancer risks by phlebotomy in blood donors were reported from non-Asian countries in America and Europe (9, 38). In addition, the patterns of serum iron levels were grossly similar between this cohort and that of NHANES (23). A fourth limitation is that, we used single point measurements of serum iron, but serum iron levels can vary with time. In fact, diurnal variation of serum iron levels has been observed in a given day (39). We compared results for those who had two tests of serum iron level and found they were highly correlated, as evidenced from the way the values were distributed and the similarity of cancer risks they demonstrated, with HR at 1.25 (95% CI, 1.16–1.35) for the first test and 1.22 (95% CI, 1.09–1.36) for the second test, in the Supplementary Table S3. Thus, the use of one single test provided sufficiently stable information for cancer risk prediction. Although serum iron is continuously metabolized by the liver, it is replenished constantly and kept

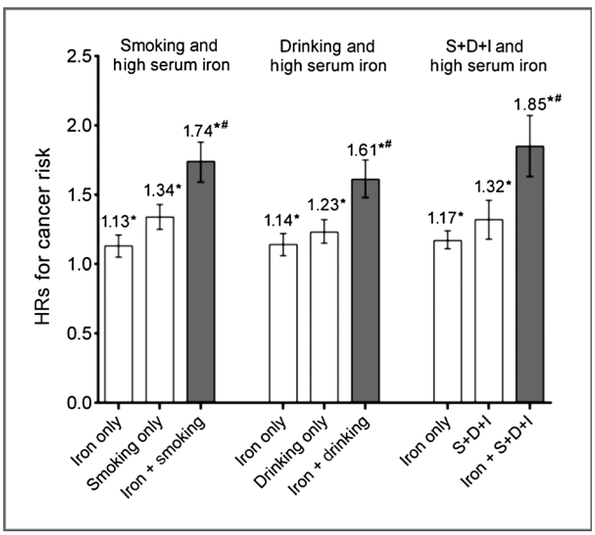

Figure 4. The effect of smoking, drinking, or inactivity, individually and in combination, with high serum iron on cancer risk in multivariate analyses. HRs were adjusted for 10 variables (Table 2); *, HR statistically significant ($P < 0.05$); #, testing for synergistic interaction statistically significant between high serum iron and risk factor(s) (RERI was statistically significant). The iron-only group, serum iron ≥120 μg/dL, but without smoking, drinking, or inactivity risk. S+D+I, smoking + drinking + inactivity. The reference group, serum iron level at 60 to 79 μg/dL.

relatively stable. Other than blood loss, the body has no mechanism for excretion of excess iron, and iron homeostasis is closely regulated within each individual. A final limitation is that the cohort came from paying participants who are considered to be at higher socioeconomic status than the average Taiwanese population. However, regarding the prevalence of high serum iron, there are no data to indicate that higher socioeconomic status had any bearing on serum iron levels.

In summary, high serum iron (≥120 μg/dL), a common occurrence observed in one third of men and one fifth of women, was associated with increased risks of incidence and mortality from all cancers combined in this cohort, and a dose–response relationship was observed. Liver cancer and breast cancer were the specific sites of significant risk increases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

Data in this research came from MJ Health Resource Center. Authorization Code: MJHRFB2014001C. The MJ Health Resource Foundation is responsible for the data distribution. Conclusions were those from authors and did not represent the views of MJ Health Resource Center.

Authors' Contributions

Conception and design: C.P. Wen, D.P.H. Hsieh, C.Y. Hsu, X. Wu
Development of methodology: C.P. Wen, M.K. Tsai, C.Y. Hsu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.A. Hsiung
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.P. Wen, J.H. Lee, Y.-P. Tai, S.B. Wu, M.K. Tsai, D.P.H. Hsieh, C.Y. Hsu, X. Wu
Writing, review, and/or revision of the manuscript: C.P. Wen, C. Wen, M.K. Tsai, D.P.H. Hsieh, H.-C. Chiang, C.A. Hsiung, C.Y. Hsu, X. Wu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.P. Wen, M.K. Tsai, H.-C. Chiang, C.Y. Hsu
Study supervision: C.P. Wen, M.K. Tsai, D.P.H. Hsieh, H.-C. Chiang

Grant Support

This work was supported in part by the Taiwan Department of Health Clinical Trial and Research Center of Excellence (MOHW103-TDU-B-212-113002).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 6, 2014; revised September 4, 2014; accepted September 8, 2014; published OnlineFirst September 16, 2014.

References

1. Gaur A, Collins H, Wulaningsih W, Holmberg L, Garmo H, Hammar N, et al. Iron metabolism and risk of cancer in the Swedish AMORIS study. Cancer Causes Control 2013;24:1393–402.
2. Cross AJ, Sinha R, Wood RJ, Xue X, Huang WY, Yeager M, et al. Iron homeostasis and distal colorectal adenoma risk in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Prev Res 2011;4:1465–75.
3. Wells BJ, Mainous AG III, Everett CJ, Gill JM. Iron, cholesterol, and the risk of cancer in an 18-year cohort. Asian Pac J Cancer Prev 2005;6:505–9.
4. Ali MA, Akhmedkhanov A, Zeleniuch-Jaquotte A, Toniolo P, Frenkel K, Huang X. Reliability of serum iron, ferritin, nitrite, and association with risk of renal cancer in women. Cancer Detect Prev 2003;27:116–21.
5. Knekt P, Reunanen A, Takkunen H, Aromaa A, Heliovaara M, Hakulinen T. Body iron stores and risk of cancer. Int J Cancer 1994;56:379–82.
6. Mainous AG III, Wells BJ, Koopman RJ, Everett CJ, Gill JM. Iron, lipids, and risk of cancer in the Framingham Offspring cohort. Am J Epidemiol 2005;161:1115–22.
7. Wu T, Sempos CT, Freudenheim JL, Muti P, Smit E. Serum iron, copper and zinc concentrations and risk of cancer mortality in US adults. Ann Epidemiol 2004;14:195–201.
8. Wurzelmann JI, Silver A, Schreinemachers DM, Sandler RS, Everson RB. Iron intake and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 1996;5:503–7.
9. Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, et al. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: results from a randomized trial. J Natl Cancer Inst 2008;100:996–1002.
10. Kowdley KV. Iron, hemochromatosis, and hepatocellular carcinoma. Gastroenterology 2004;127:S79–86.
11. Fracanzani AL, Conte D, Fraquelli M, Taioli E, Mattioli M, Losco A, et al. Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with noniron-related chronic liver disease. Hepatology 2001;33:647–51.
12. Hsing AW, McLaughlin JK, Olsen JH, Mellemkjar L, Wacholder S, Fraumeni JF Jr. Cancer risk following primary hemochromatosis: a population-based cohort study in Denmark. Int J Cancer 1995;60:160–2.
13. Foy SP, Labhasetwar V. Oh the irony: iron as a cancer cause or cure? Biomaterials 2011;32:9155–8.
14. Toyokuni S. Role of iron in carcinogenesis: cancer as a ferrotoxic disease. Cancer science 2009;100:9–16.
15. Papanikolaou G, Pantopoulos K. Iron metabolism and toxicity. Toxicol Appl Pharmacol 2005;202:199–211.

16. Huang X. Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. Mutation research 2003;533: 153–71.
17. Toyokuni S. Iron-induced carcinogenesis: the role of redox regulation. Free Radic Biol Med 1996;20:553–66.
18. Wen CP, Cheng TY, Tsai MK, Chang YC, Chan HT, Tsai SP, et al. All-cause mortality attributable to chronic kidney disease: a prospective cohort study based on 462 293 adults in Taiwan. Lancet 2008;371: 2173–82.
19. Wen CP, Wai JP, Tsai MK, Yang YC, Cheng TY, Lee MC, et al. Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. Lancet 2011;378:1244–53.
20. Andersson T, Alfredsson L, Kallberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. Eur J Epidemiol 2005;20:575–9.
21. Wen CP, Tsai SP, Chen CJ, Cheng TY. The mortality risks of smokers in Taiwan: part I: cause-specific mortality. Prev Med 2004;39:528–35.
22. Wen CP, David Cheng TY, Tsai SP, Chan HT, Hsu HL, Hsu CC, et al. Are Asians at greater mortality risks for being overweight than Caucasians? Redefining obesity for Asians. Public Health Nutr 2009; 12:497–506.
23. National Health and Nutrition Examination Survey. [cited 2013 July, 18]; Available from: http://www.cdc.gov/nchs/nhanes.htm.
24. Bonkovsky HL, Banner BF, Lambrecht RW, Rubin RB. Iron in liver diseases other than hemochromatosis. Semin Liver Dis 1996;16: 65–82.
25. Deugnier Y, Battistelli D, Jouanolle H, Guyader D, Gueguen M, Loreal O, et al. Hepatitis B virus infection markers in genetic haemochromatosis. A study of 272 patients. J Hepatol 1991;13:286–90.
26. Hann HW, Kim CY, London WT, Blumberg BS. Increased serum ferritin in chronic liver disease: a risk factor for primary hepatocellular carcinoma. Int J Cancer 1989;43:376–9.
27. Lin A, Yan WH, Xu HH, Zhu M, Zhou MY. Analysis of the HFE gene (C282Y, H63D and S65C) mutations in a general Chinese Han population. Tissue Antigens 2007;70:252–5.
28. Chang JG, Liu TC, Lin SF. Rapid diagnosis of the HLA-H gene Cys 282 Tyr mutation in hemochromatosis by polymerase chain reaction–a very rare mutation in the Chinese population. Blood 1997;89:3492–3.
29. Statistics by Country for Hemochromatosis. [cited April 10, 2014]; Available from: http://www.rightdiagnosis.com/h/hemochromatosis/stats-country.htm#extrapwarning.
30. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005;352:1769–78.
31. Chan HL, Jia J. Chronic hepatitis B in Asia-new insights from the past decade. J Gastroenterol Hepatol 2011;26:131–7.
32. Chen CH, Yang PM, Huang GT, Lee HS, Sung JL, Sheu JC. Estimation of seroprevalence of hepatitis B virus and hepatitis C virus in Taiwan from a large-scale survey of free hepatitis screening participants. J Formos Med Assoc. 2007;106:148–55.
33. Huang X. Does iron have a role in breast cancer? Lancet Oncol 2008;9:803–7.
34. Kabat GC, Rohan TE. Does excess iron play a role in breast carcinogenesis? An unresolved hypothesis. Cancer Causes Control 2007;18:1047–53.
35. Kabat GC, Cross AJ, Park Y, Schatzkin A, Hollenbeck AR, Rohan TE, et al. Intakes of dietary iron and heme-iron and risk of postmenopausal breast cancer in the National Institutes of Health-AARP Diet and Health Study. Am J Clin Nutr 2010;92:1478–83.
36. Zeleniuch-Jacquotte A, Zhang Q, Dai J, Shore RE, Arslan AA, Koenig KL, et al. Reliability of serum assays of iron status in postmenopausal women. Ann Epidemiol 2007;17:354–8.
37. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and Cancer risk–a systematic review and meta-analysis of the epidemiological evidence. Cancer Epidemiol Biomarkers Prev 2014;23:12–31.
38. Merk K, Mattsson B, Mattsson A, Holm G, Gullbring B, Bjorkholm M. The incidence of cancer among blood donors. Int J Epidemiol 1990;19:505–9.
39. Dale JC, Burritt MF, Zinsmeister AR. Diurnal variation of serum iron, iron-binding capacity, transferrin saturation, and ferritin levels. Am J Clin Pathol 2002;117:802–8.