# Association of Serum Ferritin Levels with the Risk of Stomach Cancer[1]

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## Abstract

**A group of 5908 men provided serum samples during their study examination from 1967 to 1970. After a surveillance period of over 20 years, 121 incident cases of tissue-confirmed gastric cancer were identified. Their stored sera and those of 121 matched controls from the study population were tested for serum ferritin and transferrin levels. Because of the suggested effects of previous thawing on the serum results, detailed data analyses were limited to the 46 cases and matched controls whose sera were never thawed before this study. The mean serum levels (ln ng/ml) were 5.26 for the 46 gastric cancer cases and 5.68 for their controls ($P < 0.01$). For serum transferrin, the mean levels (mg/dl) were 249.8 for cases and 254.1 for controls ($P = 0.53$). The inverse association with serum ferritin, which reflect total iron body stores, was stronger for the 21 cases diagnosed within 15 years of examination ($P = 0.02$) than for the 25 cases diagnosed after 15 years ($P = 0.15$). The limitations of the study and the implications of its findings are discussed.**

## Introduction

There is growing interest in the association of iron metabolism with cancer risk. Several recent studies have found that body iron stores are related to cancer. Positive associations have been observed for total cancer (1), as well as for lung (1, 2) and liver cancer (3), while an inverse association has been found for gastric neoplasms (4). Akiba *et al.* (4) reported that serum ferritin levels, which reflect body iron stores, were low in subjects with gastric cancer. This finding is consistent with the observation that patients with Plummer-Vinson syndrome, who have iron deficiency, have a higher risk for cancer of the upper alimentary tract, especially of the esophagus and stomach (5).

We had the opportunity to investigate further the association of serum ferritin and transferrin with stomach cancer in our cohort study of cancer among Japanese-Americans living in Hawaii. They have one of the highest risks for gastric cancer among the different ethnic groups in the United States (6).

## Materials and Methods

The subjects for this study were American men of Japanese ancestry, born between 1900 and 1919 and residing on the Hawaiian island of Oahu. They were first identified by the Honolulu Heart Program in 1965 with the use of comprehensive 1942 Selective Service draft registration files (7). Of 11,148 identified men, 8,006 (72%) were interviewed and examined between 1965 and 1968.

Of these, 7498 (94%) returned for a second examination between 1967 and 1970, at which time a serum specimen was obtained. A 20% random sample of the men had their entire serum sample sent to the USPHS Hospital in San Francisco, while the remaining 5924 men had a serum specimen stored at −20°C at the study site. There were 16 prevalent cases of stomach cancer among the 5924 men at time of examination, and these were excluded from the study.

Surveillance of this cohort to identify incident cases of stomach cancer was accomplished by continuous review of discharge records of all general hospitals on Oahu. To reduce the possibility of missing incident cases, a computer linkage file was established with the Hawaii Tumor Registry, a member of the Surveillance, Epidemiology and End Results Program of the National Cancer Institute. Based on a 19-year follow-up survey of the study subjects since their examination in 1965 to 1968, only 1.3% of the men could not be located on Oahu. As a result, the surveillance for incident cases of stomach cancer should be nearly complete.

There were 137 incident cases of gastric carcinoma diagnosed from 1968 to 1989 and confirmed by examination of tissue obtained by surgery or biopsy. Seven cases were also clinically diagnosed but without tissue confirmation, so they were excluded from the study. Sixteen of the 137 tissue-confirmed cases had an insufficient amount of serum in the freezer repository, so they were also removed from the study. The histological type of stomach cancer was determined according to the classification of Lauren (8). For purposes of presentation, the intestinal-mixed-other type will be referred to as the intestinal type which is separate from the diffuse type. There were 91 intestinal cases, 25 diffuse cases, and 5 of unknown type.

Each case was matched with one control subject selected from the study cohort based on age at exam (49 to 70) and date of serum collection. If a potential control had a gastrectomy before the serum was obtained or a hospital-based diagnosis of peptic ulcer disease before

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or after the serum was obtained, he was excluded from the study. As a result 739 subjects (13%) were removed from the control pool of 5764 men. This was done because we plan to study separately the subjects with peptic ulcer disease. Three hundred thirty-six men (6%) were also excluded because they were prevalent cases with cardiovascular disease or other cancer, and 1532 (27%) were excluded because they were diagnosed with cardiovascular disease or other cancer after their serum collection. This was done because the sera from these subjects are being used for other studies. A total of 3157 subjects remained in the pool of controls from which 121 (4%) were matched to gastric cancer incident cases. Each control was alive at the time of diagnosis of the matched case, so death was not a competing factor.

There were 46 case-control pairs for which the sera from both subjects were never thawed before preparation for analysis for this study. There were 20 pairs for which the sera had been thawed once before. The remaining 55 pairs included 9 pairs for whom the sera were thawed on two previous occasions, and the others had an uneven number of previous thawings, usually with the sera of cases having been thawed more often than those of controls.

The serum samples were sent to Nichols Institute Diagnostics (San Juan Capistrano, CA). They were coded so that the laboratory technician could not distinguish cases from controls. The Allegro ferritin immunoassay system was used to measure serum ferritin levels. It is based on the method developed by Addison *et al.* (9). The reported coefficient of variation for the test ranged from 4.2 to 7.3%. Serum transferrin was measured with the Beckman immunochemistry system using the rate nephelometry method. The reported coefficient of variation with this transferrin test is less than 5%.

The mean for serum ferritin was 305 ng/ml with a range of 11 to 3310 ng/ml for the subjects in this study. For serum transferrin, the mean was 251 mg/dl with a range of values from 135 to 389 mg/dl.

The *t* test for paired samples was used for the comparison of means between gastric cancer cases and control subjects. Due to skewed serum ferritin distribution, the comparison of means between the two groups was done after logarithmic transformation of serum ferritin levels. The log conversion of these values resulted in a range of 2.40 to 8.10 (ln ng/ml). Odds ratios for gastric cancer, based on the results of serum ferritin (after log transformation) and transferrin, were derived from conditional logistic regression methods (10). Tests for linear trend in the logit of risk were also derived from separate conditional logistic regression models through the use of grouped serum ferritin or transferrin test results (coded as 1, 2, or 3).

## Results

The results of the association between the serum transferrin test and gastric cancer are presented in Table 1. Because of concern regarding the effects of previous thawing of stored serum on serum transferrin and ferritin measurements, the data were stratified by thawing status. There were no differences in serum transferrin levels between gastric carcinoma cases and matched controls regardless of the number of past thawings.

Table 2 presents a similar comparison for serum ferritin. The 121 gastric cancer cases had a lower mean serum ferritin value than that of controls ($P = 0.05$). However, the results varied by frequency of thawing. For the 46 cases and their matched controls who had never had their serum thawed before, the serum ferritin levels were significantly lower in cases than in controls ($P < 0.01$). In contrast, there were no differences between the two groups of cases and controls whose serum specimens were thawed earlier. Because the unthawed specimens should provide more reliable results, we limited the subsequent analysis to the 46 cases and controls.

*Table 1* Mean levels (mg/dl) of serum transferrin for gastric cancer cases and matched controls by thawing status

| Thawing status | Cases | | Controls | | *P* |
|---|---|---|---|---|---|
| | No. | Mean ± SE | No. | Mean ± SE | |
| 0 thawing | 46 | 249.8 ± 5.1 | 46 | 254.1 ± 5.5 | 0.53 |
| 1 thawing | 20 | 258.1 ± 10.6 | 20 | 256.1 ± 9.7 | 0.89 |
| 2+ thawings | 55 | 243.8 ± 5.0 | 55 | 251.7 ± 5.5 | 0.28 |
| Total | 121 | 248.4 ± 3.5 | 121 | 253.4 ± 3.6 | 0.31 |

The histological distribution of the 46 cases was as follows: 35 intestinal; 9 diffuse; and 2 unknown. The mean serum ferritin levels (ln ng/ml) were 5.24 for the intestinal cases and 5.72 for their controls ($P < 0.01$). For the 9 diffuse cases, the mean level was 5.14, compared with 5.46 for their controls ($P = 0.43$).

For the 46 case-control pairs, the odds ratios by tertile groups of serum ferritin and transferrin levels were determined, as shown in Table 3. There was a significant inverse trend for serum ferritin, but no association was found for serum transferrin.

The average time (±SD) from phlebotomy to diagnosis was 15.3 ± 2.6 years for the 46 gastric carcinoma cases. We examined the association of serum ferritin and transferrin with gastric cancer in relation to the interval from phlebotomy to diagnosis (Table 4). Patients whose disease was diagnosed within 15 years after phlebotomy had a significant inverse association with serum ferritin, but not patients diagnosed after 15 years. There was no association between serum transferrin and gastric carcinoma by time interval from phlebotomy to diagnosis.

## Discussion

This investigation analyzed prospective data by using a nested case-control study design. Sera were obtained up to 20.7 years before the diagnosis of gastric cancer. Both incident cases of gastric cancer and their matched controls were from the same cohort of subjects. The findings suggest that serum ferritin levels are inversely related to

*Table 2* Mean levels (ln ng/ml) of serum ferritin for gastric cancer cases and matched controls by thawing status

| Thawing status | Cases | | Controls | | *P* |
|---|---|---|---|---|---|
| | No. | Mean ± SE | No. | Mean ± SE | |
| 0 thawing | 46 | 5.26 ± 0.11 | 46 | 5.68 ± 0.10 | <0.01 |
| 1 thawing | 20 | 5.55 ± 0.16 | 20 | 5.64 ± 0.17 | 0.73 |
| 2+ thawings | 55 | 5.23 ± 0.13 | 55 | 5.27 ± 0.11 | 0.78 |
| Total | 121 | 5.29 ± 0.08 | 121 | 5.49 ± 0.07 | 0.05 |

*Table 3* Odds ratios and 95% confidence intervals for gastric cancer by tertile distribution of serum ferritin and transferrin levels

| Serum test | No. of cases | No. of controls | Odds ratio | 95% confidence interval |
|---|---|---|---|---|
| Ferritin (ln ng/ml) | | | | |
| <5.4 | 26 | 15 | 1.0 | |
| 5.4-<6.0 | 12 | 15 | 0.5 | 0.2-1.3 |
| 6.0+ | 8 | 16 | 0.2 | 0.1-0.8 |
| *P* for linear trend = 0.02 | | | | |
| Transferrin (mg/dl) | | | | |
| <235 | 18 | 16 | 1.0 | |
| 235-269 | 15 | 14 | 1.0 | 0.4-2.3 |
| 270+ | 13 | 16 | 0.7 | 0.3-1.9 |
| *P* for linear trend = 0.55 | | | | |

*Table 4* Mean levels of serum ferritin and transferrin for gastric cancer cases and matched controls by time interval from examination to diagnosis

| Time interval (years) | Cases | | Controls | | *P* |
|---|---|---|---|---|---|
| | No. | Mean ± SE | No. | Mean ± SE | |
| Ferritin (ln ng/ml) | | | | | |
| <15 | 21 | 5.05 ± 0.17 | 21 | 5.63 ± 0.15 | 0.02 |
| 15+ | 25 | 5.43 ± 0.14 | 25 | 5.71 ± 0.14 | 0.15 |
| Transferrin (mg/dl) | | | | | |
| <15 | 21 | 255.5 ± 7.9 | 21 | 254.4 ± 7.9 | 0.90 |
| 15+ | 25 | 245.0 ± 6.7 | 25 | 253.9 ± 7.8 | 0.41 |

gastric cancer risk. There was no association with serum transferrin levels.

Because some of the serum specimens were thawed previously for other studies, we were concerned about the possible effects of thawing on study results. Consequently, the data were stratified by thawing status, with the acknowledgment that the unthawed specimens should provide the most dependable results.

A strong inverse association was found with serum ferritin among the 46 case-control pairs in which the sera had never been thawed until the time of this study. Sera that had been thawed previously and refrozen also showed that cases had lower levels of serum ferritin than controls, but the differences were not statistically significant. Previous thawing did not appear to affect the results for serum transferrin. We are not aware of any studies in the literature that have examined the effects of thawing on subsequent serum ferritin or transferrin levels.

Thirteen of the 46 gastric cancer cases, who never had their serum thawed before, also had peptic ulcer disease, cardiovascular disease, or another type of cancer. If they were excluded from the study, as were potential controls with these diseases, then 33 cases would remain. Their mean serum ferritin level (ln ng/ml) was 5.40 compared with 5.69 for their matched controls ($P = 0.09$). Because the magnitude of the inverse association was lessened in this subgroup of subjects, caution should be exercised in the interpretation of our data. It is possible that the presence of other diseases among the 13 stomach cancer cases affected their serum ferritin results.

Ferritin is the major intracellular storage protein for iron in the body (11). It is found in all tissues, and its concentration in the serum is directly related to the available iron stores. Serum transferrin is another protein that reflects iron stores in the body. Its levels in the serum are usually inversely correlated to that of serum ferritin. The Spearman correlation coefficient between serum ferritin and transferrin for the 121 cases and their matched controls was −0.18, while it was −0.24 for the 46 cases and their matched controls whose sera were not previously thawed.

Serum ferritin and serum transferrin probably reflect both long- and short-term measures of iron metabolism. They are stable over long periods of time but are affected by recent disease states (12, 13). Iron deficiency anemia leads to low serum ferritin and high serum transferrin levels. Liver disease and malignancies can result in high serum ferritin and low serum transferrin values. Elevated serum ferritin levels can also be found with hemochromatosis and acute infections (11-13).

Our findings are similar to that of another investigation that used prediagnostic serum to study the association of serum ferritin with gastric cancer. Investigators in Japan also observed an inverse association between serum ferritin and stomach cancer among 208 cases (4). There was a progressive increase in risk as the serum ferritin levels diminished, as was observed in our study. Other researchers also had data that suggested that subjects with lower body iron stores had an increased risk for stomach cancer, but only eight cases were included in their study (1). The study in Japan (4) found a weaker positive association between serum transferrin and stomach cancer which was not present in our results.

Other studies of iron metabolism and cancer have focused mainly on the effects of iron overload, partly due to the observation that iron can catalyze the production of oxygen radicals (14) which are considered to be proximate carcinogens (15). Stevens *et al.* (1) reported in a prospective study of over 8000 subjects that high body iron stores, based on the percentage of transferrin saturation, increased the risk of cancer in men, especially cancer of the lung. Selby and Friedman (2) also found a positive association with lung cancer, but more in women than in men. In contrast, Takkunen *et al.* (16) observed no association between the percentage of transferrin saturation and total cancer risk in a prospective study of over 35,000 subjects. Furthermore, lung cancer was not related to high iron stores in the study by Akiba *et al.* (4). Hann *et al.* (3) reported that increased serum ferritin levels in subjects with chronic liver disease were associated with the subsequent diagnosis of primary liver cancer. Although hepatocellular damage is related to an increase in serum ferritin, they noted that there was no simple correlation between severity of liver disease and ferritin levels in their study.

It has been suggested that the inverse association between serum ferritin and gastric cancer is related to the observation that achlorhydria diminishes the absorption of dietary iron in the gastric mucosa (4), and achlorhydria increases the risk of gastric cancer. This mechanism could account for the decrease in serum ferritin levels before the diagnosis of gastric cancer. The most common cause of achlorhydria in our study population is chronic atrophic gastritis of the distal stomach. As this process spreads proximally with age, it reduces the sur-

face area of the acid-producing mucosa (17). This process is characterized by intestinal metaplasia (18) and a reduced pepsinogen I:II ratio (17), which are both related to an increase in risk for gastric carcinoma (19, 20). Because a low serum ferritin level is a marker for iron deficiency, it should also be correlated with chronic blood loss. This condition is not uncommon with peptic ulcer and with ulcerating gastrointestinal carcinomas. If present, chronic blood loss with impaired iron absorption would further reduce serum ferritin levels.

Our data indicate that the decrease in serum ferritin levels precedes diagnosis of gastric cancer by up to 15 years. Beyond that, a low serum ferritin level is a less reliable marker of gastric cancer risk. Akiba *et al.* (4) also observed an increase in risk for subjects with low serum ferritin level up to 14 years before the diagnosis of gastric cancer. It is possible that the inverse association between serum ferritin and gastric cancer may be an indirect one, with achlorhydria being the principal factor preceding both the reduction of serum ferritin levels and the diagnosis of gastric cancer.

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