Validation of reference intervals of serum ferritin in Hong Kong

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Abstract

Background: Iron is an essential element for synthesis of haemoglobin in human body. Ferritin is the major iron storage protein and was recommended for the assessment of body iron status. However, the serum ferritin reference intervals and cut-off values adopted from World Health Organisation (WHO) and reagent manufacturers may not be applicable in local population because their recruited subjects for establishing the reference intervals were usually from western countries. The aim of this study was to establish sex-specific ferritin reference intervals from local data that would be more applicable for Hong Kong Chinese population.

Objective: This study compared the derived reference intervals and cut-off values with those recommended by WHO and reagent manufacturers.

Methods: A retrospective study was undertaken that data from 178 non-anaemic subjects (38 males and 140 females) who attended their doctors from Jan 2, 2014 to March 31, 2016, with serum ferritin concentrations, haemoglobin concentrations, gender and age were retrieved from the database of two different private laboratories in Hong Kong. After applying the inclusion and exclusion criteria, 138 (86.25%) female and 22 (13.75%) male subjects were selected for data analysis.

Results: The derived serum ferritin reference intervals for female and male subjects in this study were 6-126 μg/L and 34-271 μg/L respectively. Among female non-anaemic subjects, 28 (20.3%), 22 (15.9%) and 15 (10.9%) female subjects had serum ferritin concentrations of < 15 μg/L (WHO's cut-off), < 12 μg/L (cut-off adopted by Vuk *et al.*, 2017) and < 10 μg/L (manufacturer cut-off) respectively.

Conclusion: The lower cut-offs of serum ferritin at 6 μg/L for female subjects could be applicable for diagnosis of iron deficiency in Hong Kong Chinese population. More non-anaemic male subjects should be recruited to eliminate the gender-specific effect. Age-specific serum ferritin reference intervals were suggested for further study.

Key words: *Reference intervals, ferritin, iron deficiency*

Introduction

Iron deficiency anaemia can be indicated by a decreasing level of serum ferritin as a result of insufficient level of iron which is essential for erythropoiesis.[1,2] Serum ferritin level has been considered as a marker for chronic inflammatory diseases and associated with rheumatologic diseases.[3] High concentrations of serum ferritin were observed in pancreatic carcinoma, lung cancer, hepatocellular carcinoma and neuroblastom.[4] Moreover, liver diseases may lead to an increasing of serum ferritin level due to liver damage and inflammatory reaction.[3,5] Indeed, serum ferritin level requires the high ferritin light chain to ferritin heavy chain subunit ratio and the light chain subunit contributes a site for N-glycosylation. In addition, the ratio of glycosylated and non-glycosylated ferritin to serum concentration are changed during infection.[5]

Under normal condition, the oxygen carrying capacity of haemoglobin is maintained by the presence of iron in blood. Hence, ferritin plays an important role for iron storage in blood with a strong correlation with haemoglobin. Previous study showed that there was a strong and positive correlation between serum ferritin level and haemoglobin concentration. [6]

The reference intervals of serum ferritin concentrations used by most private and government laboratories are usually provided and suggested by the reagent manufacturers and suppliers currently. However, their recruited subjects and data usually come from western countries. Reference interval and cut-off of serum ferritin concentration provided by World Health Organisation is 15 μg/L.[7] Although the manufacturers of reagent kits and WHO provide the reference interval and cut-off for examining the serum ferritin levels, the guideline may not be applicable to Chinese population as the serum ferritin can be affected by age, gender and daily and habitual consumption.[7] In Asian Pacific regions, the mean serum ferritin concentrations were higher compared with Caucasians.[2] There was a significantly difference between Asian and Caucasians women significantly due to diabetes and haematological diseases.[8,9] Therefore, it is worthy to establish the serum ferritin reference intervals from local population and is applicable to the Hong Kong Chinese population.

This study is a retrospective study that aims to derive a reference interval and a cut-off value of serum ferritin concentration in adult male and female non-anaemic subjects based on the reference intervals of haemoglobin concentration in local population of Hong Kong. Besides, the derived serum ferritin reference intervals were compared with those provided by World Health Organization and the reagents manufacturers.

Materials and Methods

Subject data

From Jan 2, 2014 to March 31, 2016, one hundred and seventy-eight sets of data with serum ferritin concentration, haemoglobin concentration, sex and age of the non-anaemic subjects were retrieved from the database of two different private laboratories (Laboratory A and B) in Hong Kong. Non-anaemic subjects were defined as having haemoglobin concentrations within the adult sex-specific haemoglobin reference intervals, which is used as the inclusion criterion of this study. The haemoglobin reference intervals for female and male adults from Laboratory A and B are 11.5 – 16.0 g/dL (female); 13.5 – 18.0 g/dL (male), and 11.5 – 15.5 g/dL (female); 13.0 – 17.0 g/dL (male) respectively. All data sets must pass the exclusion criteria before subjected to data analysis. The exclusion criteria are (1) Outliers of haemoglobin concentrations beyond the upper or lower cut-off values of the male and female reference intervals; and (2) Outliers of serum ferritin concentrations beyond the upper or lower cut-off values of the male and female reference intervals. The serum ferritin reference intervals for female and male adults from Laboratory A and B (provided by the manufacturer) are the same [female (5 – 204 μg/L) and male (22 – 275 μg/L) adults].

Whole population and sub-group analysis of the values of serum ferritin concentrations from both laboratories were performed. The derived serum ferritin reference intervals were compared with WHO and manufacturer's intervals.

Measurement of serum ferritin concentrations

Serum ferritin concentrations were measured by the automated immunology analyzer (Abbott Architect i2000, Abbott Laboratory, IL, U.S.A.) using the Architect Ferritin reagent by both laboratories.[10] This is a chemiluminescent microparticle immunoassay in which the ferritin in samples are attached to anti-ferritin coated microparticles and the final reaction product of this immunoassay are determined and measured by the Architect optical system which detects the light signal emitted from the chemical reactions of ferritin in the samples.

Measurement of haemoglobin concentrations

Complete blood count (CBC) were measured by the automated haematology analyser on EDTA blood samples from the subjects. Sysmex XT-1800i (SYSMEX Corporation, Japan) haematology analyser was used by Laboratory A while Sysmex XN-3000L (SYSMEX Corporation, Japan) and Beckman Coulter DxH 800 (BECKMAN COULTER, U.S.A.) were used by Laboratory B. Both haematology analysers adopt a similar principle to measure the haemoglobin concentrations that a diluted blood sample was prepared by the haematology analyser. The amount of cyanmethemoglobin formed in the Ferric reaction of haemoglobin was measured spectrophotometrically in a wavelength of 540 nm, and is directly proportional to the concentration of haemoglobin in blood from

the patients.[11]

Derivation of serum ferritin reference intervals

According to the HOKLAS Supplementary Criteria No. 32 – Verification of Biological Reference Intervals from Other Sources, the Clinical and Laboratory Standards Institute (CLSI) approved guideline EP28-A3c requires a minimum of 120 reference individuals for each group (or subgroup) to establish biological reference intervals.[12] Since the data used for deriving the serum ferritin reference intervals in this study belongs to non-parametric distribution, we therefore set the lower and upper limits at 2.5 and 97.5 percentile respectively.

Results

A total of 178 adult (> 12 years old) subjects with measured haemoglobin concentrations within the reference intervals accompanied with serum ferritin results were retrieved from the database of two private laboratories in Hong Kong. The age ranges for the female, male and overall subjects were 13-84 (mean=41), 17-86 (mean=48) and 13-86 (mean=42) respectively. The testing results retrieved from Laboratory A were performed from 28th July 2014 to 5th August 2014. The testing results retrieved from Laboratory B were performed throughout the whole year of 2014 and from 2nd January 2016 to 31st March 2016. Among this batch of data, there were 140 (78.7%) female subjects and 38 (21.3%) males subjects. After applying the inclusion and exclusion criteria mentioned above, 160 subjects were included in the study and analyzed, of which 138 (86.25%) were female subjects and 22 (13.75%) were male subjects. Among female non-anaemic subjects, 28 (20.3%), 22 (15.9%) and 15 (10.9%) female subjects had serum ferritin concentrations of < 15 μg/L (WHO's cut-off), 12 μg/L (cut-off adopted by Vuk *et al*, 2017)[13] and < 10 μg/L (Manufacturer's cut-off) respectively. In contract, all male non-anaemic subjects had serum ferritin concentrations within reference interval.

Figure 1 shows the distribution between female and male among 160 non-anaemic subjects after applying exclusion criteria. The results showed that the serum ferritin concentrations were much more dispersed in male subjects.

Figure 2 shows the female serum ferritin concentrations against age. Most of the data are distributed between 5 to 160 μg/L of ferritin concentration for >40 years old female subjects. For the younger group of female subjects (<40 years old), the data was distributed between 5 - 100 μg/L of ferritin concentration.

Figure 3 compares the derived, manufacturer's and WHO's serum ferritin reference intervals for both genders. The lower reference interval of the derived female ferritin concentrations is lower than that of the manufacturer's and WHO's intervals. The serum ferritin reference intervals for female, male and combined subjects were 6-126 μg/L, 34-271 μg/L and 7-208 μg/L respectively.

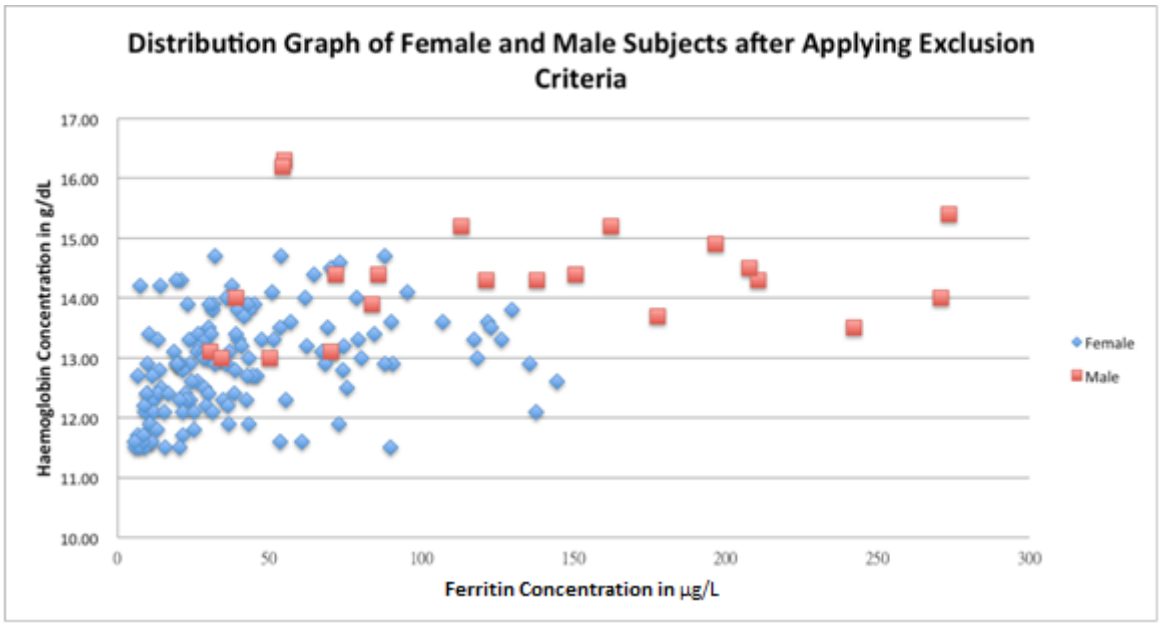

Figure 1. Distribution graph of female and male subjects after applying exclusion criteria.

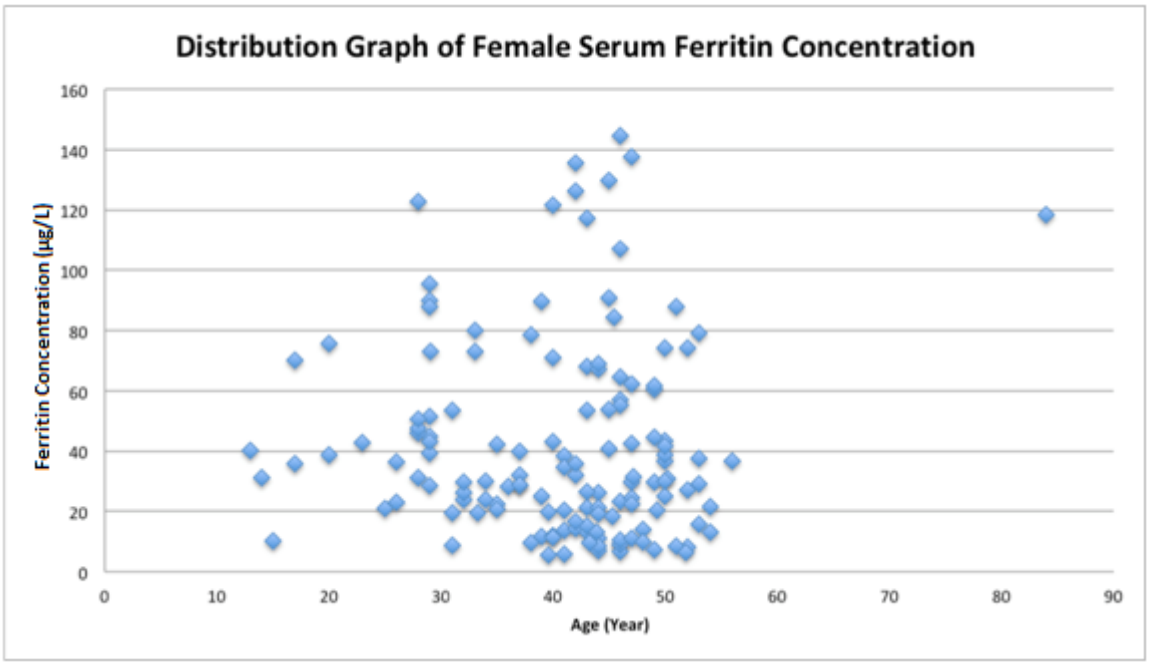

Figure 2. Distribution graph of female serum ferritin concentration

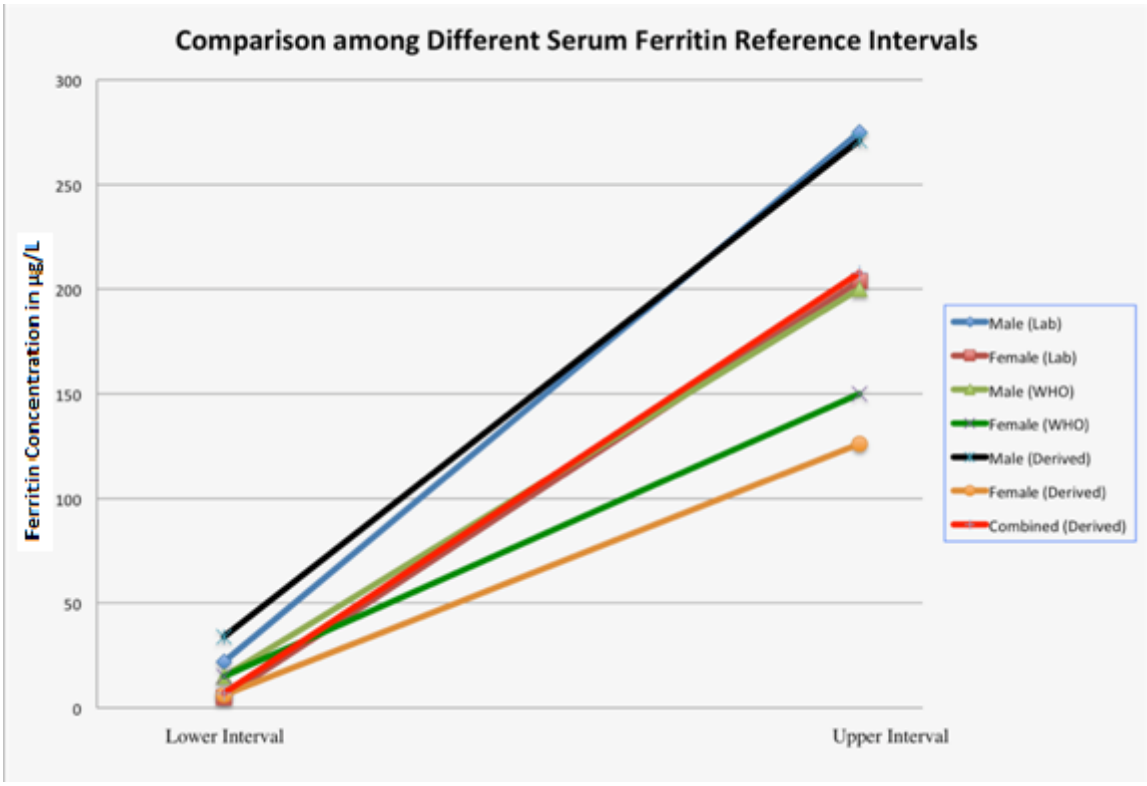

Figure 3. Comparison among different serum ferritin reference intervals

Discussions

When a laboratory adopts a reference interval for clinical testing, it has been a common practice to rely on established reference intervals from other laboratories or manufacturers for simplicity. The reference intervals suggested by manufacturers are usually based on data from the western countries. Americans and Europeans especially who are Caucasians usually have a larger body size than Chinese which may be attributed to differences in diet, lifestyle and heredity. This would lead to a higher baseline of ferritin level.[8] Therefore, the adopted reference intervals would not be appropriate for a local population. The WHO's lower cut-off of serum ferritin at 15 µg/L may not be appropriate when use in Chinese populations. This study aims to establish a local serum ferritin reference intervals in Hong Kong for the appropriate interpretation of serum ferritin results during checkup of body iron status. Moreover, there were significant differences in age and haemoglobin concentration to serum ferritin level. Thus, different haemoglobin concentrations facilitate the diagnosis or categorize the patients into anaemia group and non-anaemia group.[14]

The serum ferritin reference intervals derived in this study were 6-126 µg/L, 34-271 µg/L and 7-208 µg/L for female, male and combined subjects respectively. The range for female subjects were lower

than that of the male subjects. There are several possible reasons to explain this phenomenon. A low serum ferritin level is directly associated with a low body iron store and so factors leading to low iron level may influence serum ferritin level.[2] Menstruation in women could result in generally low serum ferritin level due to loss of a lot of blood that are rich in iron.[15] As the loss of iron cannot be replenished before next menstrual cycle, low body iron level will be sustained. Besides, heavy blood loss during childbirth may even cause iron deficiency anaemia directly.[15] Therefore, women at childbearing age could have lower serum ferritin levels than women at >40 years of age as illustrated in Figure 2. Iron supplement is necessary for women suffered from severe iron deficiency or having symptoms of anaemia.[2] Malabsorption of iron due to celiac disease may also be one of the reasons accounting for low body iron level.[16]

In Figure 3, the derived male ferritin reference interval was very close to the laboratory reference interval provided by the manufacturer. However, it is difficult to define a representative male ferritin reference interval or cut-off due to the small sample size (N=22) of recruited subjects. On the other hand, the lower cut-off of female ferritin levels in this study and the manufacturer were similar (6 μg/L vs 5 μg/L). Although there was significant difference at their upper intervals, the lower cut-off of female ferritin at 6 μg/L could be applicable in defining iron deficiency in Hong Kong Chinese population. Moreover, the actual serum ferritin baseline and levels for Hong Kong non-anaemic female subjects were lower than the WHO standard. Therefore, the derived female ferritin reference interval could be used as a reference by the physicians for report interpretation in Hong Kong Chinese female population.

The cut-off for serum ferritin concentrations recommended by WHO that reflects the status of depleted iron stores is set at 15 μg/L for female and male subjects who are five years old or older.[7] A cut-off at 12 μg/L was adopted by Vuk *et al*, 2017,[13] while it was set at 10 μg/L adopted from the current manufacturer. Unpublished data from Hong Kong Red Cross Blood Transfusion Service (HKRCBTS) reported in February this year that one in eight (12.5%) blood donors were deferred for blood donations by using the current WHO ferritin cut-off at 15 μg/L.[17] In this study, there were 28 (17.5%), about one in six among male and female subjects, non-anaemic female subjects had serum ferritin concentrations of < 15 μg/L. When focusing on female subjects only, the percentage was increased to 20.3%. If the WHO standard is adopted, the recruited subjects in this study would be classified as deferred blood donors and this would greatly reduce the donated blood supply to hospitals. In order to maintain blood ferritin level and being qualified as blood donors, it is advised to have constant intake of iron-rich foods such as meat, seafood, poultry, iron-fortified cereals, whole grains, beans, peas, and dark green vegetables. Vitamin C is also helpful for absorption of iron. Tea or coffee after

meal should be avoided to prevent interference of iron absorption.[1,18] Previous study reported that men usually have higher ferritin and haemoglobin levels than women which might be due to higher androgen level in men and loss of blood during the menstruation period and pregnancy in women.[19] Dietary monitoring would be helpful for women to maintain the haemoglobin level.

There were several recommendations regarding this study to validate the findings. Firstly, the small sample size of recruited male subjects in this study hinder the validation of a representative reference interval for ferritin cut-off value, therefore it is necessary to recruit more subjects for more in-depth assessment. Secondly, the ARCHITECT Ferritin assay might be interfered by the patient specimens which have treated with the mouse monoclonal antibodies during diagnosis. The human anti-mouse antibodies might lead to falsely positive or negative signals. Therefore, it is better to have a health questionnaire with details of medical history of recruited subjects including factors affecting serum ferritin concentrations so as to eliminate and prevent the occurrence of false results. The combination of soluble transferrin receptor and ferritin assay could be more sensitive and specific in detection of iron deficiency anaemia since soluble transferrin receptor does not increase in response to inflammation.[7,20,21] The soluble transferrin receptor to serum ferritin ratio could reflect the body iron store[8], which is a useful indicator of iron storage when assessing the Chinese population.[22] Moreover, mean cell haemoglobin concentration (MCHC) would be a sensitive early indicator of iron deficiency erythropoiesis with relatively good diagnostic efficiency.[21] Since the body iron status and serum ferritin level could vary with age, whether age is a potential confounder of serum ferritin reference levels deserves further studies.

Conclusions

In conclusion, the female serum ferritin reference interval derived in the present study could be an useful indicator in Hong Kong Chinese population. The lower cut-offs of serum ferritin at 6 μg/L for female subjects could be adopted for diagnosis of iron deficiency in Hong Kong Chinese population. More non-anaemic male subjects should be recruited to increase sample size and enhance credibility of the findings. Whether serum ferritin reference intervals could be confounded by age warrants further study.

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