**HFE gene mutation and iron overload in Egyptian pediatric acute lymphoblastic leukemia survivors: a single-center study**

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**ABSTRACT**

**Background:** Hereditary hemochromatosis gene (HFE) mutations have a role in iron overload in pediatric acute lymphoblastic leukemia (ALL) survivors. We aimed to evaluate the genotype frequency and allelic distribution of the two HFE gene mutations (C282Y and H63D) in a sample of Egyptian pediatric ALL survivors and to detect the impact of these two mutations on their iron profile.

**Patients and methods:** This study was performed on 35 ALL survivors during their follow-up visits to the Hematology and Oncology Unit, Pediatric Department, Menoufia University Hospitals. Thirty-five healthy children of matched age and sex were chosen as controls. After completing treatment course, ALL survivors were screened for the prevalence of these two mutations by polymerase chain reaction-restriction fragment length polymorphism. Serum ferritin levels were measured by an enzyme-linked immunosorbent assay technique (ELISA).

**Results:** C282Y mutation cannot be detected in any of the 35 survivors or the 35 controls. The H63D heterozygous state (CG) was detected in 28.6% of the survivors group and in 20% of controls, while the H63D homozygous (GG) state was detected in 17.1% of survivors. No compound heterozygosity (C282Y/H63D) was detected at both groups with high G allele frequency (31.4%) in survivors more than controls (10%). There were significant higher levels of iron parameters in homozygote survivors than heterozygotes and the controls.

**Conclusion:** H63D mutation aggravates the iron overload status in pediatric ALL survivors.

**KEYWORDS**

HFE; ALL; survivors; iron

**Introduction**

Hereditary hemochromatosis (HH) is a genetic disorder affecting iron metabolism resulting in iron overload-associated tissue injury. It is an autosomal recessive disorder caused by mutations in the HFE gene (on 6p21.3) [1]. It is characterized by increased iron absorption and storage, resulting in progressive and multisystem oxidative organ damage [2]. HFE gene variants correlate with body iron levels and associated with cancer risk such as childhood acute lymphoblastic leukemia (ALL) [3]. The HFE protein plays a key role in the regulation of body iron uptake through interaction with the transferrin receptor (TfR) on the plasma membrane in which it modulates the interaction of transferrin (Tf) with the TfR, thereby limiting the amount of iron that is internalized [4]. HFE is a protein of 343 amino acids that includes a signal peptide, and its extracellular domain consists of three loops with intramolecular disulfide bonds within the second and third loops which are an extracellular TfR-binding region (α1 and α2), an immunoglobulin-like domain (α3), a trans-membrane region and a short cytoplasmic tail [5]. Two mutations of the HFE gene were included as potential confounders based on their association with high iron absorption (C282Y and H63D) [6]. C282Y means substitution of tyrosine for cysteine at the 282nd amino acid position in the protein sequence. H63D is a point mutation that changes histidine to aspartic acid at HFE residue number 63. C282Y is the most common gene mutation associated with HFE-HH. Approximately 82–90% of individuals diagnosed with HFE-HH are homozygous for this gene, and this genotype has the highest risk for iron overload when inherited in this state [7]. Heterozygote mutations in C282Y/H63D account for nearly 3–8% of individuals with HFE-HH, and this genotype can result in iron overload but at lesser risk than those homozygous for C282Y [8]. Genetic mutations of H63D/H63D (homozygotes) account for approximately 1% of those with gene mutation [8]. The mechanism, by which mutations increase cancer risk, is that excess iron promotes oxidative DNA damage and free radical activity [9]. The iron-catalyzed free radical reactions cause cellular injury by lipid peroxidation, stimulation of collagen formation by activation of hepatic stellate cells, and interaction of reactive oxygen species and iron directly with DNA [10]. Altered iron metabolism affects carcinogenesis through a number of signaling pathways [11].
Objectives and aim

The aim of this work is to study the genotype frequency and allelic distribution of the two HFE gene point mutations (C282Y and H63D) in a sample of Egyptian pediatric ALL survivors and to detect the impact of these two mutations on their iron profile.

Patients and methods

This study was performed on 35 ALL survivors during their follow-up visits to the Hematology and Oncology Unit, Department of Pediatrics, Menoufia University Hospitals after completing the course of treatment. The study was held in the period from March 2015 to February 2016. Thirty-five age- and gender-matched healthy children were taken as the control group. They were volunteers recruited from schools. ALL survivors were treated by chemotherapy according to St Jude Total XV Chemotherapy Protocol [12]. None of them received radiotherapy. This study was approved by the ethical committee of Faculty of Medicine, Menoufia University. A written informed consent was obtained from the parents of all children, and oral assent was obtained from all children.

Inclusion criteria included survivors of pediatric ALL, children aged from 1 to 18 years, and survivors treated with chemotherapy only. Exclusion criteria included other types of childhood cancer, children less than 1 year or more than 18 years old, survivors treated with radiotherapy or bone marrow transplantation and patients with any disease affecting the iron metabolism.

Sample collection and preparation

Four milliliters of venous blood was withdrawn from every subject, then 2 ml was transferred into a plain tube, centrifuged for 10 min at 4000 r.p.m. The serum obtained was kept frozen at −20°C till analysis (determination of serum total iron, total iron-binding capacity (TIBC), and ferritin). Two milliliters was transferred into an EDTA tube for DNA extraction and polymerase chain reaction (PCR) at the research laboratory of Medical Biochemistry Department, Faculty of Medicine, Menoufia University. Serum ferritin levels were measured to assess the iron status of our patients.

Statistical analysis

Results were collected, tabulated and statistically analyzed by an IBM personal computer and Statistical Package for the Social Sciences (SPSS), version 20. Data were expressed in two phases: (1) descriptive phase: including the mean and standard deviation (SD) for quantitative data, frequency and percentage for qualitative data; (2) analytic phase: including U-test (Mann–Whitney test): comparison of two independent quantitative variables not normally distributed, X² (Chi-square): comparison between two or more independent qualitative variables normally distributed, T test: for comparison of two independent quantitative variables normally distributed, F-test = ANOVA test (analysis of variance) modified t-test for comparison between more than two groups and Fischer’s exact test: for comparison between two or independent qualitative variables not normally distributed.

Results

The age of children survivors ranged between 4 and 18 years. Fourteen of them (40%) were females, and 21
(60%) were males. The control group was age- and gender-matched (Table 1). The weight and BMI were significantly higher in the ALL survivors group than the control group, but no significant difference was detected between them regarding height (Table 2). Eight of our survivors (22.85%) were overweight (>85th percentile) and 10 (28.51%) were obese (>95th percentile) (Table 2). By comparing iron parameters of both survivors and controls, we found that the serum ferritin levels and TSI are significantly higher in the survivors group than controls (p value = 0.006 and 0.03, respectively), while no significant difference was detected between them regarding serum iron and TIBC (Table 3). The C282Y mutation was not present in any of the 35 survivors group or controls. The H63D heterozygous state (CG) was detected in 10 (28.6%) of survivors group and in 3 (20%) of controls, while H63D homozygous mutant allele (GG) was

Table 1. Demographic data of the studied groups.

| Parameter       | Patients | Controls | Test type | p Value |
|-----------------|----------|----------|-----------|---------|
| Age (years)     | 1.1*     | 1.1*     | >0.05     |         |
| Range           | 4–18     | 4–18     |           |         |
| Mean ± SD       | 11.01 ± 4.6 | 9.6 ± 3.3 |           |         |
| Sex             |          |          |           |         |
| Male            | 21, 60%  | 19, 60%  | >0.05     |         |
| Female          | 14, 40%  | 16, 40%  |           |         |

*U test (Mann–Whitney test).
**X², Chi square.

Table 2. Anthropometric measures of the studied groups.

| Parameter       | Patients, N = 35 | Controls, N = 15 | t test | p Value |
|-----------------|------------------|------------------|--------|---------|
| Height (cm)     |                   |                  |        |         |
| Range           | 96–177            | 98–160           | 0.94*  | 0.351   |
| Mean ± SD       | 137.4 ± 23.6      | 130.8 ± 20.8     | >0.05  |         |
| Weight (kg)     |                   |                  |        |         |
| Range           | 15–88             | 15.4–59          | 2.7*   | 0.009   |
| Mean ± SD       | 41.6 ± 17         | 28.3 ± 12.2      | <0.05  |         |
| BMI             |                   |                  |        |         |
| Range           | 13.85–33          | 16.25–26.5       | 2.6    | 0.012   |
| Mean ± SD       | 22.9 ± 6.8        | 17.6 ± 5.7       | <0.05  |         |

Bold, significant; BMI, body mass index.
U test (Mann–Whitney test).
*Significant.
Table 3. Iron profile of the studied groups.

|                  | Patients       | Controls       | U test | p Value |
|------------------|----------------|----------------|--------|---------|
| Serum ferritin   |                |                |        |         |
| Range            | 19.4–3540      | 30.99–88       | 2.8    | 0.006   |
| Mean ± SD        | 737.6 ± 99.2   | 51.6 ± 18.2    |        | <0.05   |
| Serum iron       |                |                |        |         |
| Range            | 64–167         | 58–121         | 1.4    | 0.152   |
| Mean ± SD        | 100.2 ± 36.4   | 85.8 ± 17.4    |        | >0.05   |
| TIBC             |                |                |        |         |
| Range            | 180–334        | 156–329        | 0.541  | 0.591   |
| Mean ± SD        | 273.3 ± 72.7   | 261.9 ± 55.9   |        | >0.05   |
| TSI (%)          |                |                |        |         |
| Range            | 32–50          | 32–37          | 2.2    | 0.03    |
| Mean ± SD        | 46.9 ± 21.1    | 34.7 ± 1.7     |        | <0.05   |

Bold, significant; U-test (Mann-Whitney test); TIBC, total iron binding capacity; TSI, transferrin saturation index.

Table 4. Genotype frequencies and allelic distribution of C282Y and H63D mutations among studied groups.

|                  | Patients       | Controls       | \( \chi^2 \) | p Value |
|------------------|----------------|----------------|-------------|---------|
| C282Y genotypes  |                |                |             |         |
| GG (wild homozygous) | 35 100          | 15 100         |             |         |
| H63D genotypes   |                |                |             |         |
| CC (wild homozygous) | 19 54.3         | 32 80          | 13.08       | 0.001*  |
| CG (heterozygote mutant) | 10 28.6       | 3 20           |             |         |
| GG (homozygous mutant) | 6 17.1         | 0 0            |             |         |
| Allele frequency | \( N = 70 \)   | \( N = 30 \)   | Fisher exact|         |
| C                | 48 68.6        | 27 90          |             | \( 5.1 \) |
| G                | 22 31.4        | 3 10           |             | \( 5 \) |

\( \chi^2 \), Chi-square test; S, significant; Bold, significant.

Discussion

ALL is the most common cancer in children in the USA, accounting for 26% of new cancer diagnoses at the birth to 14-year-age group. ALL is more common in boys than in girls [17]. Genetic susceptibility increases the risk of childhood leukemia or ALL. Genetic associations’ studies have found gender-specific associations (increased male-to-female ratio) including the HFE association [18]. HFE is one of the molecules that participate in iron homeostasis [16]. Most studies of iron overload in cancer survivors have been among those diagnosed as adults, and few studies have been conducted among survivors of childhood cancer [19].

We aimed to evaluate the genotype frequency and allelic distribution of the two HFE gene mutations (C282Y and H63D) in pediatric ALL survivors and to detect the impact of them on iron profile. In the current study, the weight and BMI were significantly higher in ALL survivors than in controls. This is in agreement with Zhang et al., [20] who reported that the obesity is prevalent in pediatric ALL survivors, the mean BMI \( z \) score was 0.83 which corresponds to the 80th BMI percentile, indicating a significantly higher BMI in pediatric ALL survivors than the reference population. In our study, there were no carriers for the C282Y gene mutation in the cancer survivors group or in controls. On the other hand, we detect that 45.7% of our cancer survivors had H63D gene mutation, while 54.3% did not. The allelic frequencies of C282Y and H63D are widely variable between different populations. C282Y frequency ranged from 0 to 9.9% and seen to be nearly 0% in North African population. On the other hand, the allelic frequency of H63D in different populations ranged from 0 to 20.4% [21]. These results are in line with Settin et al. [22] who studied HFE gene mutations in Egyptians with HCV infection with liver cirrhosis. They stated that C282Y was not detected among patients or controls. In this study, the H63D mutation analysis revealed that CG detected in six survivors (17.1%) and none of the controls. There is a high allele frequency of mutant genotype of H63D in survivors than controls (p value <0.05) with high incidence of mutant G allele in the survivors (31.4%) more than controls (10%), while C allele frequency was 68.6% in the survivors and 90% in the controls (Table 4). Regarding iron profile of different H63D genotypes of the studied groups, our results illustrated that the survivors of the mutant genotypes (GG and CG) were associated with the increase in the iron parameters more than non-mutant genotype (CC) and controls. There was a significant increase in serum ferritin, iron, and TIBC and highly significant increase in transferrin saturation in homozygous mutant (GG) than heterozygote (CG) and wild (CC) genotypes. In addition, a highly significant increase was found between homozygous mutant genotype (GG) and controls regarding serum ferritin and transferrin saturation and significant increase regarding serum iron, while no significant differences were detected regarding TIBC. Furthermore, no significant differences were detected between wild (CC) and heterozygote (CG) genotypes; and between controls and each of wild (CC) and heterozygote (CG) genotypes regarding all iron parameters except for serum ferritin where there was a significant increase in heterozygote genotype (CG) as compared with the controls (Table 5). No significant statistical differences were detected between male and female ALL survivors with different H63D genotypes (Table 6). There was a significant statistical increase of serum ferritin levels in male and female ALL survivors, while no significant statistical differences were detected between them regarding other iron parameters such as serum iron, TIBC, and TSI (Table 7).
Table 5. H63D genotypes

| H63D genotypes | N = 35 | N = 6 | N = 10 |
|----------------|-------|-------|-------|
| GG             | 15    | 2     | 7     |
| CG             | 6     | 2     | 2     |
| CC             | 10    | 4     | 2     |

*Bold, significant; ≥0.05; *p* < 0.05, **p** < 0.001, ***p*** < 0.001.

| Gene                   | *p* Value | *p* Value | *p* Value |
|------------------------|-----------|-----------|-----------|
| S. ferritin (ng ml⁻¹)  | < 0.05    | < 0.05    | < 0.05    |
| S. iron (μg d⁻¹)       | < 0.05    | < 0.05    | < 0.05    |
| TIBC (μg d⁻¹)          | < 0.05    | < 0.05    | < 0.05    |

| Test                  | *p* Value | *p* Value | *p* Value |
|-----------------------|-----------|-----------|-----------|
| f test, one-way ANOVA | < 0.05    | < 0.05    | < 0.05    |

| Test                  | *p* Value | *p* Value | *p* Value |
|-----------------------|-----------|-----------|-----------|
| t test                | < 0.05    | < 0.05    | < 0.05    |

Table 6. H63D genotype frequency among male and female ALL survivors.

| H63D genotypes | Male | Female | X² | p Value |
|----------------|------|--------|----|---------|
| CC             | 13   | 6      | 2.3| 0.31    |
| CG             | 6    | 4      | 2.86|        |
| GG             | 2    | 4      | 2.86|        |

Table 7. Iron profile among male and female ALL survivors.

| Test                  | *p* Value | *p* Value | *p* Value |
|-----------------------|-----------|-----------|-----------|
| S. ferritin (ng ml⁻¹)  | < 0.05    | < 0.05    | < 0.05    |
| S. iron (μg d⁻¹)       | < 0.05    | < 0.05    | < 0.05    |
| TIBC (μg d⁻¹)          | < 0.05    | < 0.05    | < 0.05    |

*Bold, significant; ≥0.05; *p* < 0.05, **p** < 0.001, ***p*** < 0.001.

| Test                  | *p* Value | *p* Value | *p* Value |
|-----------------------|-----------|-----------|-----------|
| TIBC total iron binding capacity; TSI transferrin saturation index. | | | |
out by Ling et al. [27] and compared serum ferritin, iron, and TSI values between HFE CG and CC genotypes and found no significant difference. Andreani et al. [28] reported that the HFE-H63D gene mutation is a common variant and associated with iron overload, usually in the homozygous state or in compound heterozygote individuals with C282Y mutation of HFE gene.

Study limitations
Sample size is small. Further prospective studies are thus needed to confirm our results.

Conclusion
Our study concluded that H63D mutation has a positive association with iron overload and seems to aggravate the individuals’ iron status in ALL survivors. Carriers of the mutant G allele have genetic risk factor for iron overload and carcinogenesis.

Disclosure statement
No potential conflict of interest was reported by the authors.

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