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HFE Gene Mutations as Predisposing Factors for Childhood Acute Lymphoblastic Leukaemia in Iraqi Patients

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***Abstract*—Hemochromatosis is a prevalent hereditary disorder that causes excess iron to build up in the body to dangerous levels. Hereditary hemochromatosis, also known as HFE-related hemochromatosis is carried on by changes in the *HFE* gene. Investigating the gene mutations of the *HFE* gene is a way to explore the prevalence of this disease. This study aims to determine the association between hemochromatosis *HFE* gene mutations (C282Y and H63D) and childhood acute lymphoblastic leukaemia in patients at Basra Specialized Hospital for Children and AL-sadder Teaching Hospital in the Basra governance. QIAamp DNA and Blood Mini Kit were used to isolate and identify Human genomic DNA and detect mutations in the *HFE* gene using the DNA hybridization method. In this study, the absence of the C282Y mutations in both patients and the control group was identified. However, testing DNA-based hybridization experiments revealed low detection levels of the H63D (homozygous, heterozygous) mutations; in only 12.5% of patients. The H63D (only homozygous) mutations were present in 10% of the control group. The association between patients and the control group is considered statistically significant. The HFE gene mutations (C282Y and H63D), originate in acute lymphoblastic leukaemia in childhood, thus, this study recommends complementary investigations to illustrate this case in more detail with more cases of patients and discover the hidden agents underlying these mutations.**

Keywords*— *Hemochromatosis, C282Y, H63D, ALL.

I. INTRODUCTION

Lymphoblastic leukemia has two forms, Acute lymphoblastic leukaemia (ALL) and chronic. Both are bone marrow derived cancers. The disease affects B or T-lymphoblasts with a highly frequent malignancy in childhood. Malignant white blood cells of ALL are characterized via disorderly abnormal leukocyte proliferation. Thus, leading to overproduction of lymphoblast infiltration into the blood stream and bone marrow elements resulting in a distinct disease state. Acute lymphoblastic leukemia occurs with increased frequency in patients (childhood) with Down syndrome [1], Shwachman syndrome [2, 3], Li-Fraumeni syndrome [4], Bloom syndrome [5], neurofibromatosis type I, as well as exposure to ionizing radiation, pesticides, and solvents [6-8].

The common symptoms of ALL encompass fever (as a result of leukemia or secondary infections), lethargy and fatigue (caused by anemia), joint and bone pain, and bleeding caused by low platelets [9]. The ALL symptoms include alteration in white blood cells and therefore influence the immune system and weaken the body's capacity to face infection, causing comprehensive treatment of common pathogens [10]. Acute lymphoblastic leukemia can be distinguished by relying on clinical, morphological, genetic, and immunophenotype standards as reported by the WHO of lymphoid neoplasms in 2008. ALL is further classified into three sub-types (L1-3) [11]. Patients with ALL are allocated into three risk groups: standard and moderate risk groups, both determined by sufficient premature therapy recovery; and a third group with high-risk [12].

The hemochromatosis is a recessive autosomal disorder affects iron metabolism, causing a person to absorb too much iron, thus iron accumulation led to organ damage, skin hyperpigmentation, arthritis, development of cirrhosis, diabetes mellitus, and cardiomyopathy [13]. The early symptoms for individuals with the disease include abdominal pain, weight loss, lethargy, and weakness [14, 15]. The gene responsible for hemochromatosis has been determined as a major histocompatibility complex (MHC) (class I) and is known as the *HFE* gene [16].

The *HFE* gene contains 7 exons which cover 12 kb [17]. This gene spans approximately 9,600 base pairs on chromosome 6p within the class I region of the human leukocyte antigen (HLA) complex of 4.5 Kb [18]. Exon 1 follows the peptide signal, whereas exons 2, 3, and 4 correspond to the α1, α2, and α3 regions, in that order. Exon 5 represents the transmembrane site [18]. The 5’ piece of exon 6 possesses a local stop codon and is accountable in encoding of the cytoplasmic tail. Hence, the whole-length of gene means only 6 exons [19]. The HFE protein holds 343 amino acids and possesses a peptide, a transmembrane region, binding region of an extracellular transferrin receptors (α1 and α2), a transmembrane segment, a short cytoplasmic tail, and an immunoglobulin-like α3 region [20] (Fig. 1). Molecular researches have revealed that hemochromatosis is prevailing because of a mutation in the *HFE* gene [21].

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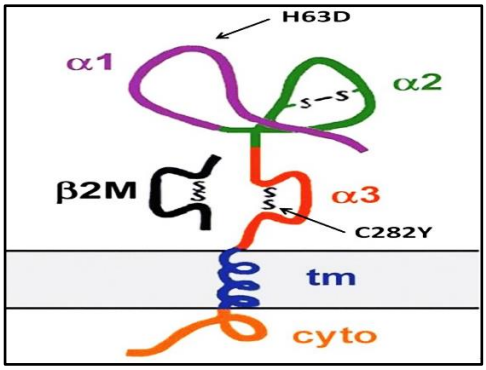

Fig. 1. The structure of HFE protein [18].

There are two major varieties of mutations linked to hereditary hemochromatosis (HH) in people of European descent; these are referred to as C282Y and H63D. Within the present more extended studies, investigations reported that the C282Y homozygote mutation has an important role in producing iron overload, while the H63D homozygote mutation provides a lower role [22]. The *HFE* gene has been shown by many studies and found to be on the 6th chromosome short arm, roughly 4.5 Kb to the HLA-A [13]. The C282Y mutation acts to disrupt disulfide bridges in the α1 and α2 extracellular domains of the *HFE* gene. The correlation between the C282Y mutation and β2 microglobulin enables the efficient transport of the C282Y mutation to the cell surface, where transferrin receptor 1 TfR1 interaction occurs. If the C282Y interaction with TfR1 is lost, the affinity of the transferrin receptor for transferrin-bound iron increases, thereby changing iron absorption [16].

II. Materials and Methods

A. *Patients and control Groups*

Samples were gathered from AL-Sadder Teaching Hospital and Basra Specialized Hospital for Children, in Basra governance. A total of sixteen blood samples among hospital patients with pediatric acute lymphoblastic leukemia were collected for DNA isolation. In addition, ten control samples were collected from healthy persons; all are without acute lymphocytic leukemia or any other disease.

B. *Genomic DNA extraction*

The human genomic DNA from both patients and control groups were used for DNA extraction and purification as well as amplification to detect *HFE* gene mutations. Human genomic DNA was extracted and purified using the QIAamp DNA Mini and Blood Mini Kit (Qiagen Germany). In addition, genomic DNA quality and quantity were measured using OPTIZEN POP Nano Bio Uv/vis spectrophotometer (DAIHAN Lab Tech, Korea).

C. *Genomic DNA amplification*

Detection of mutations in the *HFE* gene using the DNA hybridization method was performed [23]. The PCR settings were as follows: a three-minute initial denaturation step at 94 °C, 30 cycles of denaturation at 94 °C for 30 seconds each, and a 30-second annealing temperature at 54 °C. An increase of 72 °C for 30 secs (1 min for each kb of PCR product) was conducted, then a final extension at 72 °C for 3 min [24].

D. *Analysis of statistics*

Data were examined using GraphPad Prism program. The Odd ratio was used (Fisher's exact test), and 95% confidence intervals were calculated following the Bland–Altman method

III. Results

Figure 2 shows the gel of DNA hybridization of sixteen amplified DNA samples isolated from ALL patients. Figure 3 shows the DNA hybridization image analysis and Table 1 summarizes the data obtained from the image analysis in Figure 3.

It was noted that neither the control group nor any of the patients had the C282Y mutations. In comparison, the H63D (homozygous, heterozygous) mutations were detected in 12.5% of patients and the H63D (homozygous) mutations were only in 10% of the control group. Taken together, the H63D (homozygous, heterozygous) mutations were caught in both patients and the control group compared to C282Y mutations that did not show in any group.

The H63D mutations association between patients and the control group is considered to be statistically significant (P value= P<0.001; 95% CI= 2.5 to 2.5). All three positive cases, two from patients and one from the control group, show H63D gene mutations and none of the cases shows any C282Y gene mutation.

Fig. 2. Determination of C282Y and H63D genes mutations by hybridization method

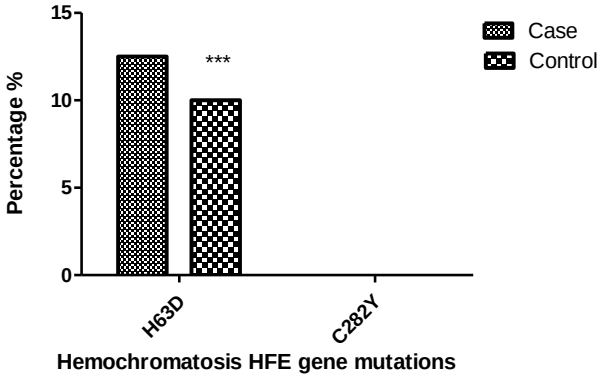

Fig. 3. The DNA hybridization image analysis of Hemochromatosis HFE gene mutations

Table 1. Distribution Hemochromatosis *HFE* gene mutations

Mutations		case		control		95% CI	Sum-Squares	P-value	
		no	%	no	%				
H63D	With	2	12.5	1	10	2.500 to 2.500	3.125	P<0.001	S*
	without	14	87.5	9	90				S
	Total	16	100	10	100				
C282Y	With	0	0	0	0				
	without	16	100	10	100				
	Total	16	100	10	100				

* S= Statistically significant.

IV. Discussion

ALL is considered the most life threatening cancers amongst children from newly born to the age of 14. Childhood leukaemia is more likely in those who have genetic susceptibility and it is more prevalent in men compared to women [25]. The purpose of this study is to determine the relationship between hemochromatosis *HFE* gene mutations (C282Y and H63D) and childhood acute lymphoblastic leukemia in patients. Various common malignancies including leukemia, colorectal cancer, and breast cancer in women show a risk link with C282Y mutation [26]. The frequency of C282Y and H63D in adults has been reported with the development of types of malignancies, including plasma myeloma, non-lymphocytic Leukemia, colon cancer, and breast cancer in women [18], and prostate cancer in men [27].

Many studies have investigated the association between cancer and hemochromatosis *HFE* gene mutations (C282Y and H63D) [28, 29][27, 28][27, 28]. The outcomes of the present study were identical to many different documented studies. A study conducted on 35 survivors with ALL in Egypt showed that none of the 35 survivors and/or the 35 controls had the C282Y mutation, whereas 17.1% of survivors, 28.6% of the patient's group and 20% of the control group had the H63D homozygous mutation [30]. There is one further published statement that reported that there is no increased frequency of C282Y in childhood leukemia patients in Finland [31].

A study conductes by Viola and coworkers has constructed a blunt report on the *HFE* gene. Adult patients with ALL had a higher frequency of (H63D mutation) than controls (P = 0.04; OR = 2.37; 95% CI = 1.05-5.36), while C282Y mutation frequency in patients with ALL and in the control group was 5% and 2% respectively [32]. This could be due to the differences in the heredity of individuals from various origins. However, a study accomplished on 36 Spanish patients C282Y and H63D mutations showed no differences between patients healthy people [33]. Another study mentioned to the relation between HFE polymorphisms and DNA damage by oxidative agents [34]. Further, a study found that C282Y mutation causes overload of serum ferritin level at 482 µg/L in female [35].

V. Conclusion

The significance of this study is to specify the hemochromatosis HFE gene mutations (C282Y and H63D) in childhood acute lymphoblastic leukaemia Iraqi patients using a DNA-based hybridization method. Accordingly, it is considered that the HFE gene mutation that is responsible for pediatric acute lymphoblastic leukaemia is found in Iraqi children patients. Thus, this study suggests complementary studies to cover this case in more detail and uncover the hidden mechanisms underlying this mutation.

Acknowledgment

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Conflict of interest

The authors declare that they have no conflict of interest.

CONTRIBUTION OF THE AUTHORS

The main project was designed by T.K and I.A. All authors performed experiments. I.A, S.J and R.W analyzed the data and wrote the manuscript and did the proof reading.

REFERENCES

[1] T. Terwilliger, and M. Abdul-Hay, "Acute lymphoblastic leukemia: a comprehensive review and 2017 update," *Blood Cancer J,* vol. 7, no. 6, pp. e577, Jun 30, 2017.

[2] T. D. Buitenkamp, S. Izraeli, M. Zimmermann, E. Forestier, N. A. Heerema, M. M. van den Heuvel-Eibrink, R. Pieters, C. M. Korbijn, L. B. Silverman, K. Schmiegelow, D. C. Liang, K. Horibe, M. Arico, A. Biondi, G. Basso, K. R. Rabin, M. Schrappe, G. Cario, G. Mann, M. Morak, R. Panzer-Grümayer, V. Mondelaers, T. Lammens, H. Cavé, B. Stark, I. Ganmore, A. V. Moorman, A. Vora, S. P. Hunger, C. H. Pui, C. G. Mullighan, A. Manabe, G. Escherich, J. R. Kowalczyk, J. A. Whitlock, and C. M. Zwaan, "Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group," *Blood,* vol. 123, no. 1, pp. 70-7, Jan 2, 2014.

[3] E. Furutani, S. Liu, A. Galvin, S. Steltz, M. M. Malsch, S. K. Loveless, L. Mount, J. H. Larson, K. Queenan, A. A. Bertuch, M. D. Fleming, J. M. Gansner, A. E. Geddis, R. Hanna, S. B. Keel, B. W. Lau, J. M. Lipton, R. Lorsbach, T. A. Nakano, A. Vlachos, W. C. Wang, S. M. Davies, E. Weller, K. C. Myers, and A. Shimamura, "Hematologic complications with age in Shwachman-Diamond syndrome," *Blood Adv,* vol. 6, no. 1, pp. 297-306, Jan 11, 2022.

[4] M. Swaminathan, S. A. Bannon, M. Routbort, K. Naqvi, T. M. Kadia, K. Takahashi, Y. Alvarado, F. Ravandi-Kashani, K. P. Patel, R. Champlin, H. Kantarjian, L. Strong, and C. D. DiNardo, "Hematologic malignancies and Li-Fraumeni syndrome," *Cold Spring Harb Mol Case Stud,* vol. 5, no. 1, Feb, 2019.

[5] A. E. Willis, and T. Lindahl, "DNA ligase I deficiency in Bloom's syndrome," *Nature,* vol. 325, no. 6102, pp. 355-7, Jan 22-28, 1987.

[6] P. Shearer, D. Parham, E. Kovnar, L. Kun, B. Rao, T. Lobe, and C. Pratt, "Neurofibromatosis type I and malignancy: review of 32 pediatric cases treated at a single institution," *Med Pediatr Oncol,* vol. 22, no. 2, pp. 78-83, 1994.

[7] R. W. Miller, "Relation between cancer and congenital defects: an epidemiologic evaluation," *J Natl Cancer Inst,* vol. 40, no. 5, pp. 1079-85, May, 1968.

[8] A. Aureli, B. Marziani, A. Venditti, T. Sconocchia, and G. Sconocchia, "Acute Lymphoblastic Leukemia Immunotherapy Treatment: Now, Next, and Beyond," *Cancers (Basel),* vol. 15, no. 13, Jun 26, 2023.

[9] M. Medinger, D. Heim, C. Lengerke, J. P. Halter, and J. R. Passweg, "[Acute lymphoblastic leukemia - diagnosis and therapy]," *Ther Umsch,* vol. 76, no. 9, pp. 510-515, 2019.

[10] J. Quessada, W. Cuccuini, P. Saultier, M. Loosveld, C. J. Harrison, and M. Lafage-Pochitaloff, "Cytogenetics of Pediatric Acute Myeloid Leukemia: A Review of the Current Knowledge," *Genes (Basel),* vol. 12, no. 6, Jun 17, 2021.

[11] F. Shahab, and F. Raziq, "Clinical presentations of acute leukemia," *J Coll Physicians Surg Pak,* vol. 24, no. 7, pp. 472-6, Jul, 2014.

[12] T. Patıroğlu, and H. H. Akar, "The Frequency of HLA-A, HLA-B, and HLA-DRB1 Alleles in Patients with Acute Lymphoblastic Leukemia in the Turkish Population: A Case-Control Study," *Turk J Haematol,* vol. 33, no. 4, pp. 339-345, Dec 1, 2016.

[13] P. L. Bittencourt, S. A. Palácios, C. A. Couto, E. L. Cançado, F. J. Carrilho, A. A. Laudanna, J. Kalil, L. C. Gayotto, and A. C. Goldberg, "Analysis of HLA-A antigens and C282Y and H63D mutations of the HFE gene in Brazilian patients with hemochromatosis," *Braz J Med Biol Res,* vol. 35, no. 3, pp. 329-35, Mar, 2002.

[14] M. S. Katsarou, M. Papasavva, R. Latsi, and N. Drakoulis, "Hemochromatosis: Hereditary hemochromatosis and HFE gene," *Vitam Horm,* vol. 110, pp. 201-222, 2019.

[15] P. C. Adams, G. Jeffrey, and J. Ryan, "Haemochromatosis," *Lancet,* vol. 401, no. 10390, pp. 1811-1821, May 27, 2023.

[16] M. Vujić, "Molecular basis of HFE-hemochromatosis," *Front Pharmacol,* vol. 5, pp. 42, 2014.

[17] J. C. Barton, and R. T. Acton, "HLA-A and -B alleles and haplotypes in hemochromatosis probands with HFE C282Y homozygosity in central Alabama," *BMC Med Genet,* vol. 3, pp. 9, Oct 7, 2002.

[18] J. C. Barton, C. Q. Edwards, and R. T. Acton, "HFE gene: Structure, function, mutations, and associated iron abnormalities," *Gene,* vol. 574, no. 2, pp. 179-92, Dec 15, 2015.

[19] W. N. Campos, J. D. Massaro, A. L. C. Martinelli, J. A. Halliwell, S. G. E. Marsh, C. T. Mendes-Junior, and E. A. Donadi, "HFE gene polymorphism defined by sequence-based typing of the Brazilian population and a standardized nomenclature for HFE allele sequences," *Hla,* vol. 90, no. 4, pp. 238-242, Oct, 2017.

[20] J. N. Feder, A. Gnirke, W. Thomas, Z. Tsuchihashi, D. A. Ruddy, A. Basava, F. Dormishian, R. Domingo, Jr., M. C. Ellis, A. Fullan, L. M. Hinton, N. L. Jones, B. E. Kimmel, G. S. Kronmal, P. Lauer, V. K. Lee, D. B. Loeb, F. A. Mapa, E. McClelland, N. C. Meyer, G. A. Mintier, N. Moeller, T. Moore, E. Morikang, C. E. Prass, L. Quintana, S. M. Starnes, R. C. Schatzman, K. J. Brunke, D. T. Drayna, N. J. Risch, B. R. Bacon, and R. K. Wolff, "A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis," *Nat Genet,* vol. 13, no. 4, pp. 399-408, Aug, 1996.

[21] N. T. Milman, F. V. Schioedt, A. E. Junker, and K. Magnussen, "Diagnosis and Treatment of Genetic HFE-Hemochromatosis: The Danish Aspect," *Gastroenterology Res,* vol. 12, no. 5, pp. 221-232, Oct, 2019.

[22] H. H. Arts, B. Eng, and J. S. Waye, "Multiplex Allele-Specific PCR for Simultaneous Detection of H63D and C282Y HFE Mutations in Hereditary Hemochromatosis," *J Appl Lab Med,* vol. 3, no. 1, pp. 10-17, Jul 1, 2018.

[23] F. Zamani, Z. Bagheri, M. Bayat, S. M. Fereshtehnejad, A. Basi, H. Najmabadi, and H. Ajdarkosh, "Iranian hereditary hemochromatosis patients: baseline characteristics, laboratory data and gene mutations," *Med Sci Monit,* vol. 18, no. 10, pp. Cr622-9, Oct, 2012.

[24] S. Mubarak, Dhafer, A. Al-Koofee, O. Radhi, A. Radhi, Jawad, J. Ismail, Zubaida, Z. Al-Zubaidi, and D. Al-Koofee, "An Optimization and Common Troubleshooting Solving in Polymerase Chain Reaction Technique," *Systematic Reviews in Pharmacy,* vol. 11, pp. 427-436, 03/01, 2020.

[25] M. S. Linet, S. Wacholder, and S. H. Zahm, "Interpreting epidemiologic research: lessons from studies of childhood cancer," *Pediatrics,* vol. 112, no. 1 Pt 2, pp. 218-32, Jul, 2003.

[26] A. Gunel-Ozcan, S. Alyilmaz-Bekmez, E. N. Guler, and D. Guc, "HFE H63D mutation frequency shows an increase in Turkish women with breast cancer," *BMC Cancer,* vol. 6, pp. 37, Feb 19, 2006.

[27] J. L. Atkins, L. C. Pilling, S. V. Torti, F. M. Torti, G. A. Kuchel, and D. Melzer, "Hereditary Hemochromatosis Variant Associations with Incident Nonliver Malignancies: 11-Year Follow-up in UK Biobank," *Cancer Epidemiol Biomarkers Prev,* vol. 31, no. 9, pp. 1780-1787, Sep 2, 2022.

[28] B. Selvaraj, S. Soundararajan, S. Narayanasamy, G. Subramanian, and S. K. Ramanathan, "Frequency of hereditary hemochromatosis gene mutations and their effects on iron overload among beta thalassemia patients of Chennai residents," *AIMS Molecular Science,* vol. 8, no. 4, pp. 233-247, 2021.

[29] J. C. Barton, J. C. Barton, and R. T. Acton, "Iron overload phenotypes and HFE genotypes in white hemochromatosis and iron overload screening study participants without HFE p.C282Y/p.C282Y," *PLoS One,* vol. 17, no. 7, pp. e0271973, 2022.

[30] F. H. El-Rashedi, M. A. El-Hawy, S. M. El-Hefnawy, and M. M. Mohammed, "HFE gene mutation and iron overload in Egyptian pediatric acute lymphoblastic leukemia survivors: a single-center study," *Hematology,* vol. 22, no. 7, pp. 398-404, Aug, 2017.

[31] Y. F. Lv, X. Chang, R. X. Hua, G. N. Yan, G. Meng, X. Y. Liao, X. Zhang, and Q. N. Guo, "The risk of new-onset cancer associated with HFE C282Y and H63D

mutations: evidence from 87,028 participants," *J Cell Mol Med*, vol. 20, no. 7, pp. 1219-33, Jul, 2016.

[32] A. Viola, L. Pagano, D. Laudati, R. D'Elia, M. R. D'Amico, M. Ammirabile, S. Palmieri, L. Prossomariti, and F. Ferrara, "HFE gene mutations in patients with acute leukemia," *Leuk Lymphoma*, vol. 47, no. 11, pp. 2331-4, Nov, 2006.

[33] M. Schneeweiss-Gleixner, G. Greiner, S. Herndlhofer, J. Schellnegger, M. T. Krauth, K. V. Gleixner, F. Wimazal, C. Steinhauser, M. Kundi, R. Thalhammer, I. Schwarzinger, G. Hoermann, H. Esterbauer, M. Födinger, P. Valent, and W. R. Sperr, "Impact of HFE gene variants on iron overload, overall survival and leukemia-free survival in myelodysplastic syndromes," *Am J Cancer Res*, vol. 11, no. 3, pp. 955-967, 2021.

[34] W. R. Gomes, P. P. Devóz, B. A. Rocha, D. Grotto, J. M. Serpeloni, B. L. Batista, A. G. Asimakopoulos, K. Kannan, F. Barbosa Jr., and G. R. M. Barcelos, "Association between Polymorphisms of Hemochromatosis (HFE), Blood Lead (Pb) Levels, and DNA Oxidative Damage in Battery Workers," *International Journal of Environmental Research and Public Health*, vol. 20, no. 4, pp. 3513, 2023.

[35] M. A. Al-Tikrity, and M. A. Yassin, "Discrepancy between Serum Ferritin and Liver Iron Concentration in a Patient with Hereditary Hemochromatosis - The Value of T2* MRI," *Case Rep Oncol*, vol. 13, no. 2, pp. 712-715, May-Aug, 2020.