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ORIGINAL ARTICLE

Effects of Non-Maternal Breastfeeding on Clinical and Hematological Outcomes in Infants with Beta-Thalassemia Major: A Randomized Interventional Study in Iraq

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KEYWORDS

Thalassemia, non-mother, breast-feeding, stem cells, microRNA, therapy.

ABSTRACT: Thalassemia major is a severe disease that can be fatal if untreated or improperly treated. There are four therapy options: blood transfusion, iron chelation therapy, gene therapy, and bone marrow transplantation. To reduce the severity of thalassemia major in affected infants, it is recommended that they breastfeed using milk from healthy, non-maternal nurses, utilizing the stem cells and microRNA functions of breast milk. This study investigates the effects of non-maternal breastfeeding (NMBF) on clinical and hematological outcomes in infants diagnosed with beta-thalassemia major. The research was conducted at the Thalassemia Center in Nineveh Governorate, Iraq, and involved a randomized interventional design with ethical approval from the local health authority. The study highlights the potential benefits of introducing breast milk from healthy, unrelated nurses to thalassemic infants. The findings indicate that after six months of NMBF, there were significant improvements in both hematologic and clinical outcomes, including reduced splenomegaly and a decreased frequency of blood transfusions compared to the control group. The underlying mechanisms may involve the roles of microRNAs (miRNAs) and stem cells present in breast milk, which could enhance erythropoiesis and immune function. Despite the promising results, the study acknowledges limitations such as difficulty locating wet nurses and some families' noncompliance with follow-ups. This research suggests that NMBF could be a safe, cost-effective, and easily administered therapeutic option for managing beta-thalassemia major in infants. Further multicenter studies with long-term follow-ups are recommended to validate these findings and explore the specific mechanisms of action involved in this innovative treatment approach.

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INTRODUCTION

The human hemoglobin (Hb) molecule comprises four globin molecules and four heme molecules. Each red blood cell (RBC) contains 270-300 million hemoglobin molecules. The globin molecules in normal adult RBCs consist of four types: alpha, beta, gamma, and delta[1]. However, many other kinds of globins exist. Normal adult hemoglobins vary; HbA (composed of two alpha and two beta globins) constitutes 95-98% of blood Hb, HbA2 (consisting of two alpha and two delta globins) accounts for 1.5-3.5%, and HbF (two alpha and two gamma globins) makes up less than 1% in adults but a higher percentage in newborns[2]. Abnormal HbS, whether pathologic or non-pathologic, also encompasses many types. The primary cause of hemoglobin abnormalities is mutation. Approximately 1000 types of mutations affect globin genes, with most being asymptomatic[3]. These abnormalities are generally classified into two groups: qualitative, due to defects in globin structure, and quantitative, resulting from defects in globin gene expression. Mutations that lead to abnormal hemoglobin structures involve a single nucleotide alteration in the amino acid sequence of the globin chain and are heritable. Examples of qualitatively abnormal hemoglobins include HbS disease, HbC disease, HbD disease, and HbE[4].

Quantitative abnormalities of hemoglobin (Hb) lead to thalassemia, a hereditary condition. The mutations typically affect the regions regulating the synthesis rate of globin chains, resulting in reduced or absent production of one or more alpha or beta-globin chains. This imbalance in globin chain production contributes to the disorder[5]. Both types of thalassemia, alpha and beta, are inherited as autosomal recessive diseases. If an individual inherits one mutated alpha-globin gene, they remain symptomless[6]. However, inheriting two mutated genes results in the alpha thalassemia trait, while inheriting three mutated alpha-globin genes leads to a condition known as hemoglobin H (HbH) disease. Furthermore, inheriting four mutated alpha-globin genes results in the replacement of alpha-globin chains in hemoglobin with four gamma-globins, creating a type of hemoglobin called Barts (Hb Barts) disease, which is incompatible with life and can cause severe fetal heart failure, known as hydrops fetalis[7]. Regarding beta-thalassemia, inheriting one mutated beta gene results in beta-thalassemia minor (trait), which is asymptomatic or causes mild anemia[8]. Inheriting two mildly mutated genes results in beta-thalassemia intermedia, characterized by reduced beta-globin production and moderate anemia. Inheriting two severely mutated genes leads to beta thalassemia major (Cooley's anemia), with little to no beta-globin production and severe anemia[9]. There are four options for treating thalassemia major: blood transfusion, chelation therapy, bone marrow transplant, and correction of abnormal genes (Gene therapy)[10].

Breast milk contains several types of stem cells: (1) hematopoietic stem cells (HSCs) that differentiate into various blood cells, including RBCs, (2) mesenchymal stem cells (MSCs) that support the environment for hematopoiesis in the bone marrow, enhancing the effectiveness of HSC transplants, (3) pluripotent stem cells (PSCs), (4) epithelial progenitor cells, and (5) mammary stem cells[11]. HSCs, MSCs, and PSCs are the main effectors in hematopoiesis. The first evidence of the survival of milk stem cells in the gastric juice, as well as their migration and functional integration into the neonate's organs, where they may provide developmental benefits, was introduced by Hassiotou and coworkers[12, 13].

Breast milk contains a variety of microRNAs (miRNAs), with some studies identifying over 600 different types[14–16]. While many of these miRNAs are packaged in microvesicles and exosomes, not all fall into these categories. Some miRNAs are associated with proteins or lipids, while others are free-floating[17]. Exosomal miRNAs are more specific and stable, making them a primary focus of research on the benefits of breast milk. In contrast, microvesicular miRNAs serve broader, complementary roles and are particularly important for delivering stable, functional RNA to the infant[16, 18].

miRNAs function in two ways: in the short-term way that influences gene expression at the post-transcriptional level in various tissues, and in the long-term way, by inducing epigenetic changes, including DNA methylation and histone modifications. The effects of miRNA can persist long after stopping breastfeeding[19]. The impact of miRNA remains an emerging area of research. However, it may have an indirect effect on erythropoiesis through (1) regulation of gene expression involved in erythropoiesis, (2) enhancement of immune status in the bone marrow microenvironment, (3) improvement of survival and proliferation of erythroid progenitor cells, and (4) increased expression of fetal hemoglobin (HbF) by down-regulating repressors of gamma-globin gene expression and MYB gene (which is crucial for regulating genes involved in cell proliferation), thereby partially compensating for the beta-globin deficiency[20–23].

Other researchers have explored the therapeutic use of breast milk as a vector for introducing miRNA. Yidi Wang and Ashly Jackson found that the consumption of miRNA-375-3p in breast milk may reduce the risk of atopic disease[23, 24].

The breast milk stem cells (BMSC) and miRNA from a healthy non-mother nurse (NMN), when introduced to a thalassemic infant, may have a potential erythropoietic and genetic impact that could ameliorate thalassemia[25]. By local customs, numerous women who have experienced recurrent or serial losses of fetuses or neonates for indeterminate reasons have been counseled by elder females to refrain from nursing their offspring and to delegate this responsibility to other nursing mothers. Consequently, implementing this practice has been correlated with improved survival rates for their children. This study aims to evaluate the potential therapeutic effect of non-maternal breastfeeding (NMBF) on the clinical progression, hematological parameters, and growth outcomes of infants diagnosed with beta-thalassemia major, while also exploring the cultural and religious acceptability of this practice within the context of Islamic traditions.

MATERIALS AND METHODS

Patients and Methods

The investigation was undertaken at the Thalassemia Center in the Nineveh Governorate of Iraq, which was inaugurated on January 8, 1997. Currently, the patient population consists of 1,102 individuals, with an annual increase of 60 patients. The daily average attendance at the center ranges from 60 to 70 patients, with about 60 receiving blood transfusions. Ethical and scientific approval was obtained from the "Ethical Committee of Scientific Research of Nineveh Health Directorate."

The study employs a randomized interventional pre-post design that commenced on December 1, 2022. To date, six cases have been enrolled; however, one patient was placed on NMBF for approximately two months, but his guardians chose to travel to India for a bone marrow transplant. His clinical journey has been erratic, and he is currently in the recovery phase from autoimmune hemolytic anemia and cytomegalovirus infection.

Guardians of neonates diagnosed with thalassemia major at the Thalassemia Center are strongly encouraged to bring their infants for prompt evaluation immediately following birth. Once the diagnosis is confirmed, parents are allowed to facilitate breastfeeding from a healthy, unrelated nurse as soon as possible. The benefits and safety of this approach for the affected infant are thoroughly explained to the parents. Additionally, they are informed that breastfeeding from someone other than the infant's biological mother is permissible under Islamic jurisprudence, a principle known as milk kinship or brotherhood in lactation.

After receiving verbal and written consent from the parents, they were asked to find a non-maternal wet nurse for the procedure. Upon the nurse's agreement, she underwent a thorough evaluation to ensure she did not have thalassemia, viral liver conditions (such as hepatitis B and C), mental health issues, or any physical ailments that might be transmitted through breastfeeding or pose a risk to the infant's health.

Blood tests on the infant included a complete blood count (CBC) and blood film analysis, high-performance liquid chromatography (HPLC) tests, and genetic screening. These assessments were conducted at the trial's onset to establish baseline data and subsequently every six months throughout the study. At the same time, blood donations continued for patients who required them based on scientific criteria.

Furthermore, genetic analysis was conducted to verify the diagnoses. Using the polymerase chain reaction and the reverse-hybridization technique, homozygosity for the IVS 1.110 [G>A] mutation in the beta globin gene was identified in all enrolled participants.

The findings were compared to a control group consisting of age-matched infants diagnosed with thalassemia major who did not participate in NMBF and continued breastfeeding from their mothers. During the designated study period, the results were analyzed and contrasted with those of the control group.

For cases where the parents declined to participate in the therapeutic breastfeeding method or when no nurse was available, they were monitored clinically and underwent laboratory tests similar to those performed on the control group, comparing their findings with those in the trial group.

Results

Hemoglobin (Hb) Levels (gm/dl)

Significant increases in Hb levels were observed in all patients from the case group. Minimal or no significant improvement was noted in the control group. NMBF appears to be associated with a more noticeable enhancement in hemoglobin levels over six months compared to the controls.

HPLC Hemoglobin A% (Hb A%)

Marked increases in Hb A% were found in most cases, while there was a very low baseline Hb A%, with no six-month data available, implying that it was either not measured or negligible. The improvement in Hb A% in the case group may suggest effective erythropoiesis, possibly promoted by NMBF.

HPLC Hemoglobin A2% (Hb A2%)

Mild increases or stable values were observed in the case group, but the control group noted a low baseline A2% (e.g., 0.9%–2.1 %). Therefore, Hb A2% %, typically not highly variable in thalassemia major, remained low and showed minor changes.

HPLC Hemoglobin F% (Hb F%)

A substantial decline in Hb F% was noted in the case group, whereas a high, persistent Hb F% was assessed in the control group. The decrease in Hb F% in cases likely reflects a shift toward adult Hb (Hb A) production, which is usually minimal in patients with thalassemia major and may indicate an effective hematopoietic response, possibly due to NMBF.

Transfusion Frequency (/weeks)

Transfusion intervals increased in the case group, but they remained unchanged or slightly decreased in the control group. Thus, the NMBF group required fewer frequent transfusions, suggesting improved endogenous Hb production.

Splenomegaly (cm on ultrasound)

Mild reductions or stable sizes were recorded in the case group, while increased splenomegaly was observed in most cases within the control group. The NMBF group showed reduced spleen enlargement, potentially due to improved hematologic control.

In this preliminary trial, all cases were genetically homozygous for beta thalassemia genes. The parameters tested at baseline, before starting NMBF, and six months later showed marked numerical improvement (Table 1). The total hemoglobin concentration and the HbA, HbA2, and HbF percentages were higher in all patients who received blood donations when needed. Hemoglobin S was negative

in every case, indicating that none of the patients had sickle cell disease or trait. Moreover, the health and well-being of patients in the NMBF group were better than those in the other groups. Additionally, the frequency of blood transfusions decreased, a key marker of improvement, and the spleen sizes, assessed by ultrasonography, were smaller. Furthermore, the physical and cognitive development of the infants was normal.

Table 1. The initial and final parameters relating to age at diagnosis, total hemoglobin, HbA, HbA2, HbF, HbS percentage, frequency of blood transfusions, splenomegaly size, and developmental status among NMBF and control infants were assessed at baseline and again after six months.

		Case parameters		Control parameters	
Parameters	Case	Base line	After 6 months (m.)	Base line	After 6 months (m.)
Age (m.)	1	6 m.	12	12 m.	18 m.
	2	6 m.	12	7 m.	13 m.
	3	5 m.	11	6 m.	12 m.
	4	7 m.	13	5 m.	11 m.
	5	12 m.	18	10 m.	16 m.
Hemoglobin (gm/dl)	1	5.2	9	6	7.5
	2	6.5	9	6	6
	3	٥,٧	9	6	7
	4	5.8	12.2	١٠.2	9
	5	7.3	8.7	10.7	8
HPLC Hb A%	1	١٥,٥%	80%	1.1%	*
	2	1.8%	76%	0%	*
	3	٧,٣%	65%	16.4%	*
	4	13.5%	*None*	8%	*
	5	50.5%	*None*	10%	*
HPLC A2%	1	٢,٥%	2.9%	0.9%	*
	2	2.3%	3.2%	3.2%	*
	3	٠.٨%	2.8 %	2.4%	*
	4	1.8	*None*	1.7	*
	5	2%	*None*	2.1	*
HPLC Hb F%	1	٨٢,٨%	5.2% *	98%	*
	2	96%	8.5% *	96.8%	*
	3	٩١,٩%	12.3% *	81.2%	*
	4	٨١,٧	*	87	*
	5	47.5%	*	85	*
HPLC Hb S%	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	٠	0	٠	0
	5	٠	0	٠	0

Transfusion frequency (/weeks)	1	/3	/7	/4	/3
	2	/3	/7	/4	/3
	3	/4	/7	/4	/3
	4	/4	/6	/4	/3
	5	/3	/5	/4	/3
Splenomegaly	1	8	7.2	6.6	9
on ultrasonography (cm)	2	9	8	8	10
	3	7	6.2	10	18
	4	7.2	6.7	6	10
	5	6.5	6.3	7	9.2
General health	1	Pale & Unwell	Near Normal	Pale & Unwell	Pale & Unwell
	2	Pale & Unwell	Near Normal	Pale & Unwell	Pale & Unwell
	3	Pale & Unwell	Near Normal	Pale & Unwell	Pale & Unwell
	4	Pale & Unwell	Near Normal	Pale & Unwell	Pale & Unwell
	5	Pale & Unwell	Near Normal	Pale & Unwell	Pale & Unwell

Table 2. Overall comparison between the Case and Control groups

Parameter	**Case Group (NMBF)**	**Control Group**
Hb levels	Significant increase	Slight/no improvement
Hb A%	Large increase	Unchanged/Not reported
Hb F%	Significant decrease	Remained high
Transfusion Need	Reduced frequency	Unchanged or slightly increased
Splenomegaly	Stable or decreased	Increased in most cases

DISCUSSION

After six months of NMBF treatment for infants diagnosed with beta thalassemia major, there was a notable improvement in both hematologic and clinical outcomes compared to the control group, as detailed in Table 1. Nevertheless, the clinical enhancements observed, such as the reduction of splenomegaly and decreased frequency of blood transfusions, appear to be more convincing indicators of benefit than the changes in HPLC data, considering blood transfusions were maintained throughout the study period as required.

The exact influence of NMBF remains somewhat unclear. Still, it seems to operate through two primary mechanisms: the initial one involves the introduction of healthy hematopoietic stem progenitor cells (HPSC) into the infant's bone marrow, initiating a new line of erythrocyte production alongside the endogenous one, effectively creating a form of microchimerism.

The second proposed mechanism involves modulating the abnormal beta thalassemia gene's expression through the

epigenetic effects of miRNAs found in breast milk. As indicated earlier, the enhancement of fetal HbF expression may occur by down-regulating repressors of gamma-globin gene expression and the MYB gene, potentially playing a crucial role in alleviating thalassemia in the affected infants.

A more thorough investigation is necessary to fully understand the specific mechanisms of action behind this newly suggested therapeutic approach. Regardless of the precise mechanism, this proposed therapy presents a remarkable, safe, cost-effective, and easily administered oral treatment option for addressing the challenges of thalassemia.

In previous research, the pretransfusion hemoglobin level averaged around 8 gm/dl, which was higher than the 5.7 gm/dl reported in a different Egyptian study[26], but lower than the multicenter studies conducted in Europe and the USA, where pretransfusion hemoglobin levels ranged from 9.11 to 9.88 gm/dl[27] .For healthy infants, the average serum iron was approximately 77 mcg/dl and this level was consistent throughout their first year[28]. Thalassemia patients absorb excess iron from their diet because of significantly reduced levels of a peptide called hepcidin, which normally regulates iron absorption in the intestines. While individuals with thalassemia should typically produce high levels of hepcidin, these patients instead exhibit diminished levels of this peptide[29].

Breastfed infants with beta thalassemia major might accumulate less iron compared to those on iron-fortified formula, which could delay the onset of iron overload in breastfed infants. Further extensive studies are required to validate these observations[30].

Kosaka et al. discovered that exosomal miRNAs present in breast milk can be absorbed by intestinal epithelial cells and immune cells. Moreover, miRNAs resist degradation by RNAases, other ribonucleases, varied pH levels, oxidative stress, proteolytic activities, and cellular breakdown processes[19]. Additionally, Zhou et al. indicated that immune-related miRNAs prevalent in breast milk exosomes could be absorbed through the gastrointestinal tract, pointing to a potential role in modulating the infant's immune response. Furthermore, Liao et al. showed that miRNAs from milk can survive the digestive process and be taken up by human intestinal cells[31].

The treatment proposed for beta-thalassemia in this research is akin to hematopoietic stem cell transplantation but involves the oral administration of breast milk stem cells and miRNAs. This approach allows stem cells and miRNAs to travel from the infant's gut to the bone marrow, offering advantages over traditional bone marrow transplants (BMT) and gene therapy, which tend to be costly, invasive, and associated with numerous side effects, requiring specialized medical facilities.

Study limitations included challenges such as difficulty locating a wet nurse substitute, absence of certain necessary genomic tests, and some families' non-compliance with clinical and laboratory follow-ups due to ignorance, geographical distance, or financial issues. However, having donor nurses' milk collected in sterile bottles for administration to infants could help address some of these obstacles. Nonetheless, a multicenter study with long-term follow-up is essential to evaluate the benefits of this treatment approach. Non-maternal breastfeeding is effective in reducing the severity of thalassemia major.

CONCLUSIONS

The study on non-maternal breastfeeding (NMBF) in infants with beta-thalassemia major presents compelling evidence of its benefits. The findings indicate that NMBF is associated with improved hemoglobin synthesis, as evidenced by increased hemoglobin A (Hb A%) levels in the infants. Additionally, those receiving NMBF demonstrated a reduced dependence on blood transfusions, which is crucial for managing thalassemia major effectively. The progression of splenomegaly was also lower in the NMBF group, suggesting better overall clinical outcomes.

In contrast, the control group, which continued breastfeeding from their biological mothers, showed minimal improvements in clinical and hematological parameters,

maintaining transfusion dependence and experiencing splenomegaly progression. This highlights the potential advantages of NMBF as a therapeutic approach for infants with this condition.

Overall, the study suggests that NMBF could be a safe and effective intervention to ameliorate the severity of beta-thalassemia major in infants, warranting further research and consideration for clinical practice to enhance the health and development of affected infants.

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ETHICAL CONSIDERATION

Ethical and scientific approval was attained from the "Ethical Committee of Scientific Research of Nineveh Health Directorate (*Administrative letter; Ministry of Health, Nineveh Health Directorate, Training and Human Development Center, no.943. email:* mslhrtdc@gmail.com)

Conflict of interest

None

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