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Evaluation of Milk Kinship Formation via Early Breast-Feeding

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Research Article

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Abstract: Consanguinity constitutes one of the major health problems worldwide, in which consanguineous marriages are associated with an increased risk for congenital malformations and autosomal recessive diseases. Although the advancement in modern biomedicine and forensic laboratories techniques continue to guide scientists to move forward to unravel many life sciences secrets, it is still ambiguous to investigate the full picture of milk kinship and its related consanguinity. Theoretically, it is possible to see such consanguinity developed from early sufficient breast-milk feeding. Thus, scientists should cooperate to investigate the problem practically to find a proper solution. This review article focuses on the alleged consanguinity that evolves from milk breastfeeding or beyond through wet nursing or milk formula feeding. Additionally, this article proposes the idea of removing milk genetic components to establish a new safe genetically free formula as a promising solution.

Keywords: Human breast milk, Bovine milk, powdered milk formulas, Breastfeeding, Wet nursing, miRNA, Blood relationship, Kinship, Siblings, and Consanguinity.

INTRODUCTION

There is no doubt that proper diet constitutes one of the important factors that contribute to healthy life. Naturally, mother's milk for a breastfeeding infant is the ultimate sufficient diet containing all the essential dietary needs for immunological and psychological developments [1,2].

Many mothers due to multiple circumstances are, however, unable to breastfeed their babies. As a result, they tend to seek for other alternatives through the wet nursing mother or manufactured milk formulas. Wet nursing remains the second choice after natural mother breastfeeding as it is compatible with human milk natural composition. Though it requires the application of some safety regulations, in addition to its restricted availability to the extensive large population. Even though humankind had developed milk banks, the availability of human natural milk does not meet its increasing demand. Therefore, scientists have developed milk formulas.

Despite the use of advanced quality control in both laboratories and milk formula factories to make formula rich in proteins, lipid, carbohydrates, vitamins, and minerals so that it will be comparable to the constituents of human milk, they still lack many biomarkers that are detectable in natural breast milk [3,4]. Recent studies revealed that milk from any source contains DNA, RNA, and other genetic components. All of these components have vital genetic roles. Milk kinship formation is considered as one of these roles. If it is approved that these components are responsible for new relationships formation between individuals, it will become a massive problem that it will lead to changing of many relatednesses and creation of subsequent consanguinity.

There is a way to test the proposed hypothesis that milk contains some genetic components which can develop new relatedness through theoretical and practical ways. Theoretically, by collecting evidence from previous studies, to understand the issue. Practically by removing genetic composition of milk and then testing the new formula on feeding babies. This article seeks to clarify the theoretical formation of new relationships between individuals after breastfeeding or with processed milk formulas. It also intends to identify the main milk components that are responsible for the formation of consanguinity.

Previous Literature

According to the literature, feeding of a newborn by wet nursing will result in the formation of a type of consanguinity, in which two infants become brothers by feeding on milk from the same mother [83-87]. This consanguinity will prevent their future marriage to each other due to their new relationship

which is regarded as incest's relationship [83]. There is a brilliant evidence of developing such consanguinity that arises from milk components, like miRNA and stem cell. If it is suggested that such consanguinity results from genetic part of milk (specifically, miRNA) [23], then it will become a massive worldwide problem, because it will be extended to involve milk sources other than human milk such as bovine milk or powdered milk formulas that contain these genetic components [84].

Newborn Feeding Sources

Milk whether it is human breast milk, bovine milk or manufactured milk formulas, is a multi-ingredients liquid that provides infant by nutrition and growth factors which helps in regulation of many immune processes [1,2]. Naturally, human milk is considered as the optimal biological infants' nutrition that contains all biochemical factors needed for life [5-8]. Human milk contains suitable dietary factors such as nutritional agents, cytokines, chemokines and hormones [9,10], and milk whey regarded as its main fraction [11].

Mother's own milk is the standard choice for every infant in order to meet all dietary requirements. Sometimes, and due to some reasons, that stand as an obstacle in front of this choice, milk substitutes like donor human milk is regarded as a second choice in front of bovine milk formulas [12,13]. Animal milk and other milk formulas remain the last choice for many considerations [14].

Long time ago, humankind used to introduce wet nurse for breastfeeding of infant instead of natural mother's milk in case of inability of the mother to feed her babies [88]. Many cultures used this route as a substitutive way with confined restrictions. For instance, some ancient Arabic tribes made rules for the chosen wet nurse, such as her wisdom, cleverness, strength, health, and absence of diseases, the possibility of consanguinity formation between people, the risk of disease inheritance, physical and mental qualities, emotions, and many others. They believed that her characters will be transferred to her offspring via breastfeeding. Due to many modern habits, it becomes difficult to find a super wet nurse. The increasing demand for a wet nurse in many societies has forced many countries to develop more organized ways to deal with this type of feeding, like organized milk banks [89]. Human milk banking offered a substitutive solution for many suffering mothers as a suitable natural feeding way away from milk formula products and other milk feeding methods. Beside human breastfeeding, wet nursing, and milk banking, the worldwide use of bovine milk and their products as a natural source of nutrition has been known for a long period [15]. This was done through sterilization and pasteurization in the old method (directly), or through complex multi-step process manufacturing method (infant formulas) [90]. Despite the role of infant formula as an alternative source of nutrition to an infant, the consequences of unknown and hidden genetic components with a lot of genetic traits are still ambiguous.

Milk Composition and Genetics

Generally, human milk composed mainly of macronutrients, micronutrients, bioactive components, growth factors and immunological factors [16], in addition to a large number of bacteria [17]. All of these components indicate that human breast milk is more potent than infant formula. 1. Macronutrients: these are proteins (whey and casein), fats and lactose. 2. Micronutrients: these constitute vitamins and minerals. 3. Bioactive components: like Milk Fat Globulin (MFG) and Vascular Endothelial Growth Factor (VEGF). 4. Growth factors: such as Epidermal Growth Factor (EGF), Insulin-like Growth Factor (IGF), Erythropoietin (EPO) and Somastatin. 5. Immunological factors: cytokines, chemokines, and oligosaccharides.

Human breast milk consists mainly of epithelial cells that contain both RNA and DNA. These cells constitute or form about 50-90 % of cell types in the milk [18], for that, breast milk is considered as a library for maternal genetic information. Genetically, one of the main constituents of human milk is nucleotides, which is considered as conditionally essential substances. Human milk has a significant value of nucleotides. On one hand, many studies showed their beneficial effects on human flora [19,20]. On the other hand, these nucleotides act as a bank for building and repair of genetic makeup. But it is assumed that the main reason behind the milk kinship formation and subsequent consanguinity depends on milk genetic composition hypothesis [21,22]. These genetic components include miRNA and stem cells [23-25], beside some organics that regulate epigenetic roles and gene transcription. Stem cells beside miRNAs are the important factor information of new family relationships. A study done by Baier *et al*. [26] revealed that genetic components like RNA were responsible to exert functions on the human body. We can denote this with the microchimerism process.

Maternal-fetal microchimerism is the presence of maternal cells or DNA in offspring [27]. This was suggested by an experiment done on mice by Weiler *et al.* [28], in which experimental mice were fed milk that contained marked maternal cells. The cells were shown to be found in offspring tissues. Additionally, to mention an important part of the interaction between miRNAs and stem cells, some miRNAs are specifically expressed in stem cells to play important role in the control and differentiation of cells [29].

Micro RNA

Micro RNA is short (19-24 nucleotides) endogenous, noncoding [30-32], small regulatory RNA molecules that have specific activity on target messenger RNA (mRNA) to play specific roles [24,25,33]. They are bioactive non-coding RNA molecules that regulate gene expression and control rate of protein production in cells by interfering with molecular machinery required for translating mRNA into proteins [21,23]. These constituents are present in many types of milk sources such as human milk [21,23,34,35], bovine milk [36-40], pigs milk [41] and rats milk [42]. This miRNA mainly found in cell, fat and skim milk [43]. MiRNA microarray results reported by Kosaka *et al*. [21] showed that there are many types of miRNA in human breast cells indicating that they have several functions other than their immunological functions.

In general, miRNA is present in many forms in the most body fluids, but the main thing is its presence in the milk and its whey. Also, mRNA is found in rat milk whey [37-39, 42], and human milk [44,45], but its presence in bovine milk is yet to be confirmed.

One of the main characters of these miRNA is that they are stable in acidic media and in the presence of RNase enzyme [37,21,38,46]. Furthermore, the presence of RNase in our body fluids endangers the presence of intact RNA [46] and thus the presence of miRNA suggests that these segments are resistant to this enzyme. Kosaka *et al*. [21] confirmed that miRNA in breast milk was resistant to low pH, thus it can tolerate low (acidic) pH of infants' gastrointestinal tract. Due to the resistance of miRNA in milk to harsh conditions, so its consumption by infant will not be affected by denaturation processes. Additionally, freeze-stored breast milk is not affected by low temperature. Notably, the resistance of miRNA to several harsh conditions such as the effect of RNase, freeze-thawing cycles and low pH have been reported in the literature [21]. Owing to the fact that miRNA is very resistant to harsh conditions, they can thus be used in human milk as a biomarker, such us using miRNA in the detection of lactation performance and mammary glands health [5,47,48].

Different Milk Types Contain miRNA

The presence or detection of miRNA in commercial milk products is incredible [36,38]. There are few studies that have reported the presence of miRNA in raw and processed bovine milk. As issued in milk scientific reports, miRNA is present in infant formula; these constitute about one-tenth the level of unprocessed milk [36-38].

Both human and animal sources of milk were found to be rich in miRNA quantities as free or inside exosomes [49], and even in milk formulas (bovine milk-based or soy-based) [36]. Seven milk-associated miRNAs in natural raw milk, commercial milk fluid, and infant formula-powdered milk were detected in milk. These are miR-26a, miR-26b, miR-200c, miR-21, miR-30d, miR-99a, and miR-148a. Arntz *et al*. [50] suggested that miRNA in bovine milk can be shown in the bloodstream of consumer and it has many roles in different tissues and cells. An important fact is that skimmed bovine milk contains 245 miRNAs [36], some of them were similar to those found in human milk.

A study by Alsaweed *et al*. [51] reported that infant formulas were low in human miRNA content; this was done as a comparison between human milk and milk formula. Regardless of its quantities, it is still an indication of the presence of miRNA in milk formula. The study also disclosed that different types of human miRNA differ clearly from those found in maternal blood. Generally, this study concluded the infant formula contained 45 mature human miRNAs. Another important thing is that 33 miRNAs were common between bovine milk-based formula and human milk. Chen *et al*. [36] performed miRNA expression profile for seven milk-specific miRNAs for quality control of milk-related products, they found a significant amount of those miRNAs, but they were lower in fluid milk and formula than that of raw milk. Although the expression levels of those seven miRNAs in the studied 25 milk formulas were lower than expected, it remains as an indicator of their roles. On one hand, Alsaweed *et al*. [51] found that the content of human mature miRNA was to a lesser extent in both bovine milk-based formula and soy-based formulas. On the other hand, this will not neglect their functions despite their small or minute quantities.

Although some steps in infant formula preparation have gone into excluding and discarding of cell debris as well as milk fat layer via centrifugation and pasteurization, this process does not get rid of miRNA quantity in the milk [52,53].

Mechanism of miRNA Action

A scientific report by Kosaka *et al*., [21] reveals that miRNA which is a genetic material that is transferred from human to human by a method that differs from sexual reproduction method. A brilliant main feature of newborns gut is the character of its greater permeability facilitates miRNA inside microvesicle transport, thus enhancing exosomes absorption [71]. Then these vesicles pass from intestinal epithelial cell to lymphatic system via transcytosis process and finally to circulation [72,73].

Many studies revealed that RNA from extracellular portion of cells can be present in many forms, like exosomes and microvesicles as well as in ultra-centrifuged supernatants of whey fraction [54], but the majority of RNA are present in exosomes [55-59]. Furthermore, studies have shown that human breast milk miRNA are stable *in vitro* which suggests their presence in microvesicles or exosomes [60]. The

majority of miRNA are synthesized in lactocyte (mammary cell) epithelium [61]. Exosomes are small vesicles that contain and protect miRNA against many conditions [67], this miRNA is then secreted into milk fluid by milk-producing cells. Also, they contain many biomolecules such as proteins. These exosomes (lactosomes or microvesicles) composed of dense sucrose and contain single-stranded 60s and 70s RNA that are associated with reverse transcriptase enzyme [68-70].

According to Irmak *et al*. [62], microvesicle protrudes from the mammary epithelial cell directly via apocrine mechanism or indirectly via crescent of Milk Fat Globulin (MFG). Endocytosis is the main process by which human or bovine milk exosomes are taken up by intestinal cells [63]. Initially, it starts with vesicle uptake by the cell, which is ready for harboring state and prepares its compartments for hosting by caveolar internalization route. By endocytosis, vesicles form caveolae inside the cytoplasm of fetal cells, these vacuoles with their included microvesicles translocate specific proteins to block breakdown by a lysosomal pathway that degrade these vehicles. This route has an advantage of the protection of vesicle from endosomal-lysosomal network [64]. Caveolar route guides vesicle to be directed to the endoplasmic reticulum (ER) by mean of microtubules or to the nucleus after their detaching from the plasma membrane [65]. Some microvesicles use lipid-rate-dependent internalization pathway especially those vesicles that do not have caveolar invagination ability. Vacuoles attach to endoplasmic reticulum to liberate microvesicles and their genetic constituents by the help of specific proteins, such as Hsp 70 and Cyclophilin A. Released RNA combine with reverse transcriptase enzymes and toward the nucleus. The latter enzyme begins its role to integrate RNA into fetal DNA. Finally, these vesicles (exosomes) bind and fuse into cell membrane (CM) of target cells, then they release their contents [37,66].

Eventually, miRNAs that are found in food have been transported to adult's circulation through the stable process via gastrointestinal tract (GIT) too [74]. Obvious evidence comes from the presence of miR-159a in the bovine milk-based formula that is the miRNA specific for the plant but which was detected in circulation. It indicates that the animal was fed plant-based nutrition or due to the addition of plant parts to bovine formula during manufacturing [74]. Another evidence from recent studies shows that osa-MIR-168 which is miRNA from rice could be incorporated to exert function *in vivo* by its detection in human and mouse sera [74]. Furthermore, miRNA can be transferred to the plasma through consumption of bovine milk. It is reported that miR-29b increased in plasma after 4 to 8 hours and then fell to normal levels after 24 hours [26].

Human macrophages can take up exosomes that are present in human breast milk [58]. This shows that exosomes from other species can be taken up by human cells. A study performed by Izumi *et al*. [54] showed that bovine raw milk-derived exosomes can only be taken up by human macrophages, but it is still unknown whether macrophage cells are the only cells that can take up these exosomes or not.

Effect of miRNA on Recipient Organism and its Roles

As stated above, miRNAs are highly stable in infant's formula, thus indicating their possible effects even in such milk types. Results shown by Izumi *et al*. [54] confirmed that miRNA contained in bovine milk has a tendency to affect human cells. To confirm the effect or action of miRNA on the recipient, for instance, miRNA from rice were found in the blood. The miRNA molecules help in the production of liver protein in mouse [74].

Recent studies clarified that miRNAs have a main role inside the cell to control gene expression. Many different types of miRNA were detected in human breast milk, but their exact function is still unknown. However, the expression pattern of miRNA contained in different milk samples from the same mother differs slightly with time [21].

Retrotransposons constitute the main transposing elements that participate in the formation of the wide extent of mammalian genome via reverse transcription [75,76]. There are two main types of these transposons, retroviral-like and nonretroviral retrotransposons. Retroviral-like retrotransposons have the ability to integrate into and out of chromosomes by a mechanism that resembles that used by a retrovirus (i.e. through DNA replication and cell division) following these steps: A. transcription of entire transposon to get DNA copy. B. translation of this transcript to mRNA by the host cell. C. host cell then encodes for transcriptase enzyme. D. transcriptase enzyme makes DNA copy of RNA. E. DNA double strands molecule integrate itself into the chromosome by the action of integrase enzyme [76]. Nonretroviral retrotransposons constitute the large part of our genome, as they follow different mechanism as follow: A. incorporation of RNA copy to target DNA to act as its template. B. endonuclease-reverse transcriptase enzyme complex to cleave DNA at sites of insertion to leave free 3'-OH DNA end to be used for reverse transcription process. C. this generates single copy DNA strand that is linked to host DNA directly. D. DNA single copy will result in the formation of double-stranded DNA [76].

So, it is confirmed that the formation of infant retrotransposons from the feeding of an infant with breast milk mediates the transfer of maternal mRNA and miRNA to infant through microvesicle and

processed by reverse transcription to incorporate such genetics to infant's genome.

Additionally, it is important to know that milk fat globules (MFG) contain about 14000 high-quality RNA transcripts located in their cytoplasmic crescents [44]. This conscriptome reflects reverse transcription capability of RNA content.

Consanguinity Problem

Current studies reveal these epigenetic materials (especially miRNAs) have many effects on gene expression of an infant by their inheritable character. The result is the production of new phenotype that will undergo further gene expression to other new epigenotypes in the next generations. Therefore, feeding of the many infants who are not blood-related by the same mother will result in sharing of the same epigenotype and thus lead to a fate similar to that which occurs in consanguineous marriage.

Recently, some evidence confirmed that previous epigenetic roles are inherited through the meiotic process; this is called transgenerational epigenetic inheritance. This means that many features of one generation will affect the phenotype of subsequent generation [77-79]. All of these epigenetic inheritance features have also been earlier reported [80-82].

Different cultures in many societies believe that a lot of genetic characters are inherited to offspring via breast feeding. For instance, in the Islamic religion, infant feeding creates new relationships between infant, the wet nurse, and her offspring. This is known as milk kinships. As stated in THE HOLLY QURAN (An-Nisa Surah, Ayah 23), this new form of relationship which prevents the future marriage of this individual to his wet nurse and to her children as they have become siblings through breastfeeding to form blood relationships.

On the hand, the alleged transfer of many genetic traits and developing of new relationships between individuals via early milk feeding makes the issue very sensitive world widely, especially if we identify such consanguinity beyond wet nurse feeding and extend it to milk formula feeding. This article tends to clarify this issue according to available scientific knowledge and the proposal that focuses on the presence and transferring of genetic materials from different milk sources and their effects on the host (infant).

DISCUSSION

Previous reviews have disclosed some of hidden milk genetics secrets, such as its genetic components and their functions. These genetic constituents include mainly miRNA in addition to DNA and stem cells. Many studies revealed that these genetic ingredients (mainly miRNA) were found to be present in human breast milk as well as other milk sources like bovine or manufactured processed milk formula. Some studies have also confirmed their effects on recipient's genome. MiRNAs have unique features and characters which enable them to exert effects. These effects may vary according to some contributing factors, like milk type, its quantity, recipient's age and maternal state. The main effect of their action is their responsibility to modify recipients' genome to form a new family relationship which is called milk kinship. From a theoretical aspect, it is possible to conclude their roles to create such relatedness and to see subsequent consanguinity as a consequence of milk kinship via breastfeeding or even milk formula. The further practical investigation should be taken seriously to explore milk genetic composition and their roles specifically as a factor for developing milk kinship and its related consanguinity. To understand the issue, scientists of Biomedicine and its related subjects (Pharmaceutical Biotechnology, Cell Biology and Genetics), Forensic medicine and Pediatrics medicine, along with advanced practical techniques have enabled us to know many genetic secrets, like identifying the genome, as well as DNA fingerprints, DNA mutation and transformation, all of which are now available to investigate people blood relationships and subsequent effects of these relationships. Thus, we can apply the understanding to study genetic changes in an individual. Nowadays, all facilities are available at laboratories level to reach the level of manufacturing of gene-free milk. If it is proven that milk genetic constituents such as miRNA, stem cells, DNA and RNA are present, then it is obligatory to remove these ingredients from processed milk to obtain safe milk formula. In other words, can we imagine the impact of early feeding on a milk of source other than biological mothers such as a wet nurse, bovine and powdered milk formula on the modification of newborn's genetic makeup, as well as the development of new family relationships (milk kinship)?

CONCLUSION

It is suggested that milk from any source contain genetic components that have many roles. This genetics can be absorbed and detected on the recipient. Their characters enable them to interfere with the fetal genome to create new boundaries of relatedness via milk kinship theory. These days, it is available to use forensic medicine tools to test and investigate both of milk genetics composition and fetal genome changes, pharmaceutical biotechnology to set up new gene-free milk formulas, and biomedicine to understand the issue and to develop a safe solution to be applied to practical life. The cooperation of scientists in related fields is recommended to enhance scientific libraries.

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