COMPARATIVE ANALYSIS OF THYROID PROFILE IN BREAST-FED AND BOTTLE-FED INFANTS

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ABSTRACT

Background: Hormonal derangements of infancy are among preventable diseases. Therapeutic administration of synthetic hormone is an expensive option. Breastmilk being optimized with all the required nutrient is a natural option. Breastfed infants are, therefore, supposed to have less variations in hormonal levels.

Objectives: To measure thyroid in infants exclusively on breast Feeding and formula milk feeding.

Methods: This study comprised of eighty infants of both genders divided into two groups, one exclusively breastfeeding and other on formula fed milk. Blood samples were analyzed for thyroid function tests through chemiluminescent immunoassay technique. Differences if any was statistically calculated by applying One-Way ANOVA/Independent T Test through GraphPad Prism 8.0. A p-value ≤ 0.05 was considered significant.

Results: Serum T3 mean values in all groups ranged from 1.43 ng/mL to 1.65 ng/mL. T3 mean values were numerically higher in formula-fed female group. There was no significant statistic difference present among various groups, with a p value of 0.083. Serum T4 mean values in all groups ranged from 104.95 ng/mL to 119.8 ng/mL. T4 mean values were numerically higher in breast-fed female group. There was no significant statistic difference present among various groups, with a p value of 0.423. Serum TSH mean values in all groups ranged from 2.56 µIU/mL to 2.83 µIU/mL. TSH mean values were numerically higher in breast-fed male group. There was no significant statistic difference present among various groups, with a p value of 0.632.

Conclusion: There is no significant statistical difference present in thyroid hormones in breastfed and formula fed groups.

Key words: Breastfeeding; Formula fed or (Bottle fed); Thyroid Stimulating Hormone (TSH); 3,5,3′-Tri-Iodothyronine (T_3); Thyroxine (T_4); HMO (Human milk oligosaccharides); BMI (Body Mass Index); IF's (Instant Formula's); TH (Thyroid Hormone); ABEI-Labelled (Amino-Butyl-Ethyl-Isoluminol-Labelled); IgA (Immunoglobulin A)

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INTRODUCTION

Human breast milk is recommended as the optimal source of nutrients for newborns up to six months due to its personalized, plentiful nutritious elements, immunological protection, and bioactive elements.[1] Breast milk provides newborns with essential nutrients such as water, carbohydrates, lipids, proteins, vitamins, minerals, and biologically active molecules such as oligosaccharides, cytokines, and growth factors.[2] Breast milk provides newborns with essential nutrients and

immune system components, protecting them from immune-mediated dysfunction and diseases, making it a crucial preventive measure against the immune triggered disorders.[3] Breast milk from a newborn's mother is declared as the top dietary source by the World Health Organization (WHO) for newborns' viability, well-being, and existence. It is a secure, innate, nourishing and continuous unending food for newborns.[4] Breastfeeding is crucial for a newborn's first six months to ensure optimal growth throughout their growing years.[5] Breastfeeding provides essential nutrients and calories for the early lifespan of newborns, enhancing intelligence, reducing obesity and risk of developing diabetes in future.[5]

Macronutrients and micronutrients are equally included in breast milk composition, and their proportions fluctuate frequently depending on the different variables, for instance, maternal and newborn health, mother's dietary intake, environmental and hereditary variables.[6] Human milk oligosaccharides (HMO) are highly rich biologically active compounds in breast milk and having numerous benefactory activities for example assisting in growth of useful microorganisms, anti-inflammatory properties, immunological evolving properties and activation of intestinal barrier activities.[7] Recently, HMOs have been produced synthetically in the form of additional supplements to infant milk formula for newborns who cannot be breastfed. Thus, promoting development while delivering defense from various diseases during the initial age of lifespan.[8] Clinical research has been done to determine how maternal BMI during and after pregnancy affects the makeup of HMOs and how it influences the newborn's development.[9,10] Additional mother's statistics, such as nutritional data, should be collected in upcoming studies in order to gain an understanding of how the lipidome of breast milk affects the growth of newborns.[11] It has been found that newborns ingestion of isoleucine, leucine, and aromatic amino acids has a positive correlation with the maternal body mass index (BMI).[10]

There are numerous breastfeeding benefits, including breast milk having the most optimal nutrient concentration. It also consists of several antibodies against many diseases, due to which the immunity of infants improves. Working mothers with long duty hours have less chance to breastfeed their infants due to a shortage of time. On the other hand, formula feeding provide a balanced nutritional diet, which can fulfill the requirements of infants of malnourished mothers and mothers with deficient milk production. For newborns whose mothers breastfed regularly have a lower chance of breast carcinoma, ovarian cancer, hypertension, diabetes mellitus, and strokse. Breast cell derived stem cells and their potential contribution in neonatology is upcoming field of research for researchers and scientists.[12] Breast milk is having bioactive constituents responsible for anti-viral, anti-inflammatory and immunoregulatory impacts.[13] The bioactive constituents of human breast milk contain required microorganisms, human milk oligosaccharides (HMOs), dietary polyunsaturated fatty acids, immunoglobulins and lactoferrin.[14] HMO regulate the immune response by upgrading the intestinal epithelial barrier functions.[15] The breast cell derived stem cells takes part in management of various certain pathological conditions.[12] Breastfeeding limitations include malnourished mothers, breast malignancies, hormonal defects in mother's incapable of breastfeeding. IF's have been created to mimic the nutritional advantages of breast milk. The objectives are to yield identical results for optimum progress, growth, immunological maturation, and metabolic process programming. The growth patterns and body composition progress of IF's continue to vary from those of breastfed newborns regardless of constant advancements, which may increase the possibility of obesity for formula-fed newborns.[16] Formula-fed limitations include weak bonds between mothers and infants. Infants on formula feeding have low immunity due to the non-availability of IgA secretion in their feed.

Thyroid hormones (TH) are endocrine hormones which controls almost each cell of the human body. Hypothyroidism and hyperthyroidism, the deficiency and excess respectively demonstrate the function of thyroid hormone on fetus growth, carbohydrate metabolism, lipid metabolism, overall development and progress of cardiac, vascular, neuronal and reproduction systems.[17] A comprehension of the usual embryology and physiology of the embryonic and newborn thyroid hormone level will assist in analyzing an infant for thyroid diseases.[18] Thus, this study was designed to assess the levels of thyroid hormones in newborn infants fed on breastmilk or formula milk (Bottle milk). Eighty blood samples were drawn with the written consent. Forty samples were collected from breast fed infants further divided into twenty male and twenty female breastfed groups. Forty samples were collected from formula fed infants group further divided into twenty male and twenty female formula fed groups. Assessment of thyroid function tests was done by commercially available kits by using chemiluminescent immunoassay method. This preliminary study aims to provide foundational insight through a comparison between breast-fed and bottle-fed infants by analyzing their thyroid hormone levels in early infancy, supporting the cost-effective prevention of developmental disorders through early detection and the potential for treatment with synthetic hormones; it also highlights the need for more comprehensive research to

determine whether and how breast-feeding and bottle-feeding influence infant endocrine development.

METHODS

Materials used: Disposable Syringes (5cc), Butterfly Branula, Serum Cups, Spirit Swabs, Centrifuge Machine, Gel Activator Vials, Maglumi 800 Chemiluminescence Immunoassay Analyzer, Maglumi T3 Commercially Available Kit, Maglumi T4 Commercially Available Kit, Maglumi TSH Commercially Available Kit.

This study design was a cross-sectional comparative type of study design. This study was performed in the Biochemistry Laboratory, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore (UOL). The duration of Study is one year.

Sample Size: A total of 80 infants of both genders were studied. Each group was comprised of 40 infants of both genders, 20 female and 20 male infants, using the following formula:

$$N = z^2 \times p\,(1\text{-}p) \div e^2$$

The sample size was calculated by the formula[19] by taking the prevalence of 41%.[20] The marginal error has been increased to 11% due to the constraints of the cost and budget.

Sampling criteria:

Inclusion criteria: The inclusion criteria required infants of both genders from birth until one year.

Exclusion criteria: Exclusion criteria required infants with mothers having hormonal disturbances and endocrine co-morbidities.

Exclusion criteria required infants with congenital deformities and known pathologies such as Down syndrome, CP child (cerebral palsy), and thalassemia.

Sample Collection: Informed written consent was obtained from parents and guardians residing in the city area of Lahore. A questionnaire was filled out, asking about the mother's health, the child's wellbeing, and feeding habits. A Five ml blood sample was drawn from either arm or pedal veins, whichever was clearer and more accessible, using a butterfly needle attached to a 5 mL syringe. The blood was immediately transferred to yellow-capped gel activator tubes. The tubes were centrifuged at 4000 rpm for 10 minutes. The separated serum was stored at -20ºC in three separate aliquots or portions in serum cups until further analysis.

Serum Parameters Estimation:

Serum T3 (Triidothyronine)/Serum T4 (Thyroxine)/ Serum TSH (Thyroid Stimulating Hormone):

The frozen serum samples were thawed. 40 microliters of serum were uploaded to the Maglumi 800 automated chemiluminescence immunoassay analyzer. The given reagents provided by the manufacturer were also loaded. The sample displacing solution, ABEI-labelled anti-T3 monoclonal antibody/ABEI-labelled anti-T4 monoclonal antibody/ABEI-labelled anti-TSH monoclonal antibody, buffer, and T3 antigen-coated magnetic microbeads/T4 antigen-coated magnetic microbeads/TSH antigen-coated /TSH antigen immobilized on the magnetic microbeads for a limited number of binding sites on the ABEI-labelled anti-T3 antibody/ABEI-labelled anti-T4 antibody/ABEI-labelled anti-TSH antibody, forming immunocomplexes. After precipitation in a magnetic field, the automated analyzer decanted the supernatant and then performed a wash cycle. Subsequently, the light signal initiated in a chemiluminescent reaction was measured by a photo multiplier as relative magnetic microbeads were mixed thoroughly and incubated inside the automated analyzer. T3/T4/TSH present in the serum sample competes with T3 antigen/T4 antigen light units (RLUs), which were inversely proportional to the concentration of T3/T4/TSH present in the sample. The T3 concentration calculated in the sample was expressed as ng/mL.[21] The T4 concentration calculated in the sample was expressed as ng/mL.[21] The TSH concentration calculated in the sample was expressed as microIU/mL.[21]

Statistical data analysis: Data was entered and analyzed using SPSS version 27. Numerical data, including thyroid function tests levels, was analyzed using the one-way Anova/Independent T test through GraphPad Prism 8.0. The result was expressed as the mean ± SD. A p-value ≤ 0.05 was considered significant.

RESULTS

The serum obtained from breastfed and formulafed infant were centrifuged and analyzed giving following results. The age of infants of both genders ranged from birth to one year.

Serum T3 (Triidothyronine): Serum T3 Triidothyronine was measured using the chemiluminescent immunoassay method, and values are presented in Table 1. The mean values were slightly numerically higher in the formula-fed groups. The values in female groups were also generally higher than those of male groups. The highest value was 1.65 ng/mL in formula-fed females, followed by 1.63 ng/mL in formula-fed males. The mean values of breastfed females were 1.46 ng/mL, and those of breastfed males were 1.43 ng/mL. There is no significant statistic difference present among various groups, with a p value of 0.083.

Serum T4 (Thyroxine): Serum T4 (Thyroxine) was measured using the chemiluminescent immunoassay method, and values are presented in Table 1. The mean values were slightly numerically higher in the breastfed groups. The values in female groups were also generally higher than those of male groups. The highest value was

119.8 ng/mL in breastfed females, followed by 116.23 ng/mL in breastfed males. The mean values of formula-fed females were 116.06 ng/mL, and those of formula-fed males were 104.95 ng/mL. There is no significant statistic difference present among various groups, with a p value of 0.423.

Table 1. Mean values of thyroid hormones presented as mean ± sd in breastfed and formulafed males and females infants

Group Name	B Males	F Males	B Female	F Females	ANOVA
T3	1.43 ± 0.33	1.63 ± 0.33	1.46 ± 0.26	1.65 ± 0.41	0.083
T4	116.23 ± 31.33	104.95 ± 17.13	119.8 ± 33.82	116.06 ± 33.03	0.423
TSH	2.83 ± 0.973	2.56 ± 0.745	2.61 ± 0.684	2.80 ± 0.759	0.632

Serum TSH (Thyroid Stimulating Hormone): Serum TSH was measured using the chemiluminescent immunoassay method, and values are presented in Table 1. These mean values were generally higher for breastfed male group than breast-fed female group. The highest value was 2.83 µIU/mL found in breast-fed males, followed by 2.80 µIU/mL in formula-fed females. The mean values were 2.61 µIU/mL in breast-fed females. The lowest mean values were 2.56 µIU/mL found in formula-fed male groups. There is no significant statistic difference present among various groups, with a p value of 0.632.

DISCUSSION

Breastfeeding is well-recognized for its numerous health benefits, including optimal nutrient composition and the presence of maternal antibodies that enhance infant immunity. It has also been associated with long-term health benefits for mothers, such as reduced risks of breast and ovarian cancers, hypertension, and metabolic diseases. However, breastfeeding may not always be feasible—particularly in mothers who are malnourished, have hormonal imbalances, or suffer from conditions like breast malignancy or idiopathic low milk production. In such cases, formula feeding serves as a viable alternative, providing balanced nutrition that can support healthy infant development. Nonetheless, formula feeding lacks the immunological components found in breastmilk, such as secretory IgA, which may contribute to reduced immune protection in formula-fed infants.

The current scientific research suggests the nutritive value of HMOs, lipid contents, and proteins of recent IF's (Instant Formula) bear more resemblance to the structure of HMOs, lipids, and proteins of breast milk. For newborns who are incapable of being breastfed, IF's have been created to mimic the nutritional advantages of breast milk. The objectives are to yield identical results for optimum progress, growth, immunological maturation, and metabolic process programming. Understanding this transition and the hormonal profiles associated with different feeding types can provide critical insights into neonatal care and endocrine development.

Additionally, the discussion must address a broader, systemic concern: the absence of standardized, region-specific reference ranges for hormone levels. As highlighted by Zadey (2021), biological and environmental variability across populations can significantly affect hormone baselines.[22] Establishing geographically relevant hormone reference ranges is essential to ensure accuracy in diagnosis, effective treatment planning. T3 levels, while lower than those in newborns, also show a steady rise, reaching approximately 45 ng/dL by term.[23] Similarly, TSH levels increase modestly throughout gestation, reflecting the maturing hypothalamic-pituitary-thyroid (HPT) axis. LaFranchi et al. (2021) observed significant variations in serum T4 levels, indicating potential variability across populations and sample characteristics. Our study findings are in line with those of de Cock et al. (2017), based on his study, there were no statistically significant difference observed in the serum TSH levels of newborns.[24]

After birth, thyroid hormone levels undergo significant physiological shifts, especially in the first hours of life due to abrupt changes in the thermal environment and cessation of placental support. These changes are crucial for triggering postnatal adaptation, making early thyroid screening vital. Premature infants, in particular, may have immature HPT axis development and insufficient maternal hormone transfer, placing them at greater risk of hypothyroxinemia. While this study did not reveal statistically significant differences between feeding types in thyroid hormone levels, it adds to a growing body of evidence suggesting the need for more nuanced, large-scale investigations. Future research should include longitudinal designs, larger sample sizes, and consideration of maternal health status, environmental factors, and genetic influences. Establishing standardized regional hormone reference ranges will be essential to accurately evaluate endocrine health in infants and optimize early intervention strategies.

CONCLUSION

No statistically significant difference was found in the hormonal levels of the thyroid profile in this study. It was quite possible we might find differences if this study is repeated with the advancing age of infants. Differences might also be found if the study is repeated

with mothers stratified into proper nutritional categories. No statistically significant difference in this study is probably due to the very preliminary nature of this study. So, exploration with further study design is required.

Declaration of Helsinki: The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, and in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals. The informed consent was obtained for experimentations with human subjects. The privacy rights of human subjects were observed.

ETHICAL APPROVAL

Ethical approval was granted by the Board of Advance Studies and Research, the University of Lahore, vide reference No REG/019/23/9608 dated: 25/11/2023

CONFLICT OF INTEREST:

Authors declare no conflict of interest.

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AUTHOR'S CONTRIBUTIONS

SAK: Concept, design, manuscript writing, data collection
AA: Concept, design. data analysis, critical review
SA: Data analysis and interpretation
AHS: Statistical analysis, Literature review
ALL AUTHORS: Approval of the final version of the manuscript to be published

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