LABORATORY METHODS USEFUL IN THE ANALYSIS OF HUMAN BREAST MILK AND MILK POWDER SAMPLES

BOGDAN NEAMTU¹, OVIDIU TIŢA², MIHAI NEAMTU³, MIHAELA TIŢA⁴, MIRELA HILA⁵, IONELA MANIU⁶, LUCA LIVIU RUS⁷

Keywords: laboratory methods, breast milk, milk powder formula, infant immunity

Abstract: Human breast milk contains substances with anti-infective roles, immunoglobulins, cells involved in immune responses, prebiotics, and important nutrients for infants. Immunonephelometry, polyacrylamide gel electrophoresis, ultrasound spectroscopy can be used to analyze the composition of human milk. In our research, we have examined the sensitivity of these methods in the quantification of lactose, fat, and pH of human breast milk and the sensitivity regarding evaluation of proteins, immunoglobulins (A, G, M) concentrations in both human breast milk (52 samples) and milk powder (48 samples). The results showed the appropriateness of using the ultrasonic spectroscopy only for the analysis of the fat content, pH and lactose. For milk samples immunogram (breast milk and milk powder) immunonephelometry can be considered as a reference method. Polyacrylamide gel electrophoresis remains the method of choice in assessing protein concentration. Breast fed infants have superior immune protection in particular by IgA titers and β-globulins.

INTRODUCTION

Breast milk contains substances with antimicrobial and antiviral roles, immunoglobulins, cells involved in immune responses, prebiotics. Breast milk is composed of fats (AG-fatty acids, PUFA polyunsaturated fatty acids-AG), proteins (casein, α-lactalbumin, albumin, β-lactoglobuline, IgA, IgG, lactoferrin, lysoyme), carbohydrates (lactose, oligosaccharides), minerals (calcium, phosphorus, sodium, potassium, chloride), bioactive factors.(1,2,3,4,5)

Although it has a complex composition, human milk can be easily divided by centrifugation into three major parts, namely soluble whey, casein, the mycelia of globules of milk fat (MFGs floating). Breast milk contains different components necessary for newborn for growth and development. Among these components, specific proteins in milk and plasma proteins, such as β-casein, k-casein, α-lactalbumin, serum albumin, lactoferrin, lysoyme, immunoglobulins A, C3, C4 (complement fractions) that have significant nutritional and immunological functions.(1,2,3,5)

Numerous methods have been reported as tests used for the analysis of the composition of human milk: 1.immunonephelometry, 2 polyacrylamide gel electrophoresis, 3.chromatography of the proteins in a liquid medium, 4.ion exchange chromatography, 5. Kjeldahl method. 6. Mojonnier, Gerber, and Babcock methods 7. Ultrasonic Spectroscopy.(6)

Nephelometric and turbidimetric methods of analysis are based on the phenomenon of diffusion or absorption of light by solid particles or colloids by measuring the intensity of scattered light flux by solid particles in a solution. Immunonephelometry is based on conventional nephelometric quantification of the scattered light of antigen-antibody complexes formed during the immunoprecipitation reaction liquid phase, and is usually used for the determination of human serum proteins including IgA, and fractions of the complement C3, C4. This technique allows the measurement of IgA, complement fractions in mature human milk C3, C4 with precision and accuracy.(6) Ultrasonic spectroscopy, a non-destructive analytical technique measures the parameters of low-energy ultrasonic waves propagating through the sample analyzed. It allows probing intermolecular forces in the sample, providing new information on the structure.(7) It is used to analyze the content of milk fats, proteins, lactose, physico-chemical parameters such as density, freezing point, added water, pH, temperature and conductivity in fresh milk (cow, sheep buffalo, goat).(8) An interesting approach with implications concerning the improvement of the milk powder formula composition is to test the reliability and suitability in evaluating the composition of breast milk samples from infants.
hospitalized, using predominantly methods of laboratory analysis of human serum (immunonephelometry, polyacrylamide gel electrophoresis) and methods of analysis of milk samples of animal origin (ultrasonic spectroscopy).

METHODS

The proposed study aimed at analyzing the differences in composition between globulin fractions α1, 2, β, γ, immunoglobulins A and G studying samples of milk and milk powder using immunonephelometry and polyacrylamide gel electrophoresis, but also the ultrasonic analyzer performance in the analysis of breast milk samples (used in the food industry for testing milk samples of animal origin). To this end, we have collected biological samples from nursing mothers admitted in Pediatric Clinic of Sibiu in order to study the composition of breast milk and milk powder. We have tried to establish regarding the analysis of samples of human milk and milk powder, the limitations of laboratory methods used in our clinic just for the analysis of serum from blood samples until now.

The samples were collected aseptically and with local antisepsis (areola disinfected, using sterile gloves) following the protocol: 52 control samples (skin disinfected) for verification, 52 breast milk samples and 48 samples of infant formula. We have used sterile gloves and sterile containers for milk. Samples were transported on ice to the laboratory and kept in a refrigerator (-20 °C) for further analysis.

Biological samples from human milk and milk powder were subjected to centrifugation at 3,000 rotations/minute for 20 minutes. The supernatant represented by fat was separated and the filtrate was used to determine the Ig G, A, M and to perform electrophoresis of the proteins. Electrophoresis was carried out with the device Genio S, using the cellulose acetate film at a basic pH. The determination of immunoglobulins (IgA, F, G) was carried out on samples of the filtered Hitachi device 912 by immunoturbidimetric method (readings at λ=340 nm). Evaluation of physical and biochemical parameters of human milk was performed using an ultrasonic analyzer. Pearson correlations, statistical tests (Independent Sample Test) were studied in accordance with the objectives of the study.

RESULTS AND DISCUSSIONS

In samples of breast milk protein the content was 3.45 g/dl (mean) with a minimum of 3.24 g/dl and a maximum of 3.58 g/dl after analysis of data provided by ultrasonic Ekomilk Total Analyzer. The same samples were also tested on electrophoresis device Genio S and the values were expressed in g/dl (mean was 1.021 g / dl, with a minimum of 0.268 g / dl and a maximum of 1.48 g/dl). Basically there was a discrepancy between the measured values offered by ultrasound analyzer and the electrophoresis device, regarding the determination of protein. The literature shows that proteic macronutrients of mature breast milk vary between 0.9 and 1.2 g/dl and differs from one mother to another during lactation and nutritional status.(5,6,9) In a recent study, Zachariassen et al (2013).(10) have shown a high degree of variability of protein content in 736 breast milk samples collected from mothers with premature infants. It has been described even a much wider range from 1.06 g / dl to 2.96 g / dl. The data on the concentration of proteins in colostrum showed values of 2% greater than the transitional milk (5%) and mature milk (1%).

The fat content of breast milk samples had an average of 3.85 g / dl with a minimum of 0.79 g/dl and a maximum of 7.64 g/dl. In the specific studies, values of 3.6 g / dl with a minimum of 2.2 g/dl and less than 5 g/dl colostrum are reported.(5,6) Fat content evaluation was performed only on ultrasonic analyzer and it is appropriate. The content of lactose

CLINICAL ASPECTS

Figure no. 1. Globulin synopsis breast milk / milk powder

Figure no. 2. IgA levels comparison breast milk / milk powder

\[ \alpha_1, \alpha_2, \beta, \gamma \]

\[ \gamma \]

\[ \beta \]

\[ \alpha_1, \alpha_2, \beta, \gamma \]

\[ \gamma \]

\[ \beta \]
CLINICAL ASPECTS

Table no. 1. Breast milk protein levels

<table>
<thead>
<tr>
<th>Device</th>
<th>Origin of the proteins</th>
<th>No.</th>
<th>Mean g/dl</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>Total proteins of breast milk</td>
<td>52</td>
<td>1.022</td>
<td>0.2687</td>
<td>0.58</td>
<td>1.48</td>
<td>0.7375</td>
</tr>
<tr>
<td>Analyzer</td>
<td>Total protein in breast milk ± 0.2</td>
<td>52</td>
<td>3.456</td>
<td>660E-02</td>
<td>3.24</td>
<td>3.58</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Table no. 2. Breast milk fats

<table>
<thead>
<tr>
<th>Device</th>
<th>Biomarker type</th>
<th>No.</th>
<th>Mean g/dl</th>
<th>Standard deviation</th>
<th>Min</th>
<th>Max</th>
<th>Percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasonic Analyzer</td>
<td>fats ± 0.1%</td>
<td>52</td>
<td>3.8521</td>
<td>1.8869</td>
<td>79</td>
<td>7.64</td>
<td>2.72</td>
</tr>
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Table no. 3. Breast milk lactose and pH levels

<table>
<thead>
<tr>
<th>Device</th>
<th>Biomarker type</th>
<th>No.</th>
<th>Mean g/dl</th>
<th>Standard deviation</th>
<th>Min</th>
<th>Max</th>
<th>Percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasonic Analyzer</td>
<td>Lactose ± 0.2 %</td>
<td>52</td>
<td>5.021</td>
<td>1.219</td>
<td>4.76</td>
<td>5.25</td>
<td>4.95</td>
</tr>
<tr>
<td>Ultrasonic Analyzer</td>
<td>pH ± 0.02</td>
<td>52</td>
<td>7.042</td>
<td>660E-02</td>
<td>6.8</td>
<td>7.16</td>
<td>7.01</td>
</tr>
</tbody>
</table>

Table no. 4. Statistical significant correlations of bioactive factors in milk and milk powder

<table>
<thead>
<tr>
<th></th>
<th>Breast fed infants</th>
<th>Formula fed infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human milk</td>
<td>Positive Pearson correlations</td>
<td>β- globulins with μA</td>
</tr>
<tr>
<td>Formula</td>
<td>Negative Pearson correlations</td>
<td>β- globulins with α1, γ</td>
</tr>
</tbody>
</table>

Mean concentration of IgA in breast milk samples (83.71 mg / dl, SD = 38.03) was significantly higher (p < 0.05) than the average of IgA concentration in milk powder samples (9.45 mg / dl, SD = 2.71). Mean β-globulin for breast milk (M = 29.95%, SD = 15.73) was significantly higher than the mean of β-globulins in milk powder (M = 14.30%, SD = 10.52). No significant differences between groups for γ-globulins (p = 0.697 > 0.05) were, meaning that the mean of γ-globulins in breast milk (M = 14.71%, SD = 16.02) was not significantly higher than the mean of γ-globulins for milk powder (M = 13.68%, SD = 9.93). Mean of α1 globulin in breast milk (M = 11.89%, SD = 9.41) was significantly lower than the α1 globulin in milk powder (M = 16.94%, SD = 10.85). Mean of α2 globulin in breast milk (M = 18.41%, SD = 11.87) was significantly higher than the mean for α2 globulin in milk powder (M = 12.81%, SD = 8.18). It can be noticed that there is a superior immune protection to those fed with formula. Breast fed infants with respiratory infections have a superior immune protection to those fed with formula.

CONCLUSIONS

Analyzing the data presented, the following conclusions are emerging consistent with the objectives of the study on the analysis of samples of breast milk and milk powder:

1. Polycrylamide gel electrophoresis is suitable for the determination of proteins in breast milk, and analysis devices based on ultrasound spectroscopy used in food technology require specific adaptations.
2. Immunonephelometry can be successfully used to analyze serum samples resulted from the preparation of milk and milk powder respectively.
3. Devices based on ultrasonic spectroscopy used in the food industry can be successfully used in the analysis of fat content, pH and lactose.
4. Breast fed infants with respiratory infections have a superior immune protection to those fed with formula.

REFERENCES

7. http://www.scientistlive.com/content/9474,


