Mother’s milk and hydrogen peroxide

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Original Article

Introduction
Mother’s milk is an established and healthy food source of energy, proteins, vitamins and minerals. In addition to its value as a nutrient source, interest has arisen in the ability of milk to kill bacteria, viruses, fungi and in its anti-amoebic properties. Several milk proteins have anti-microbial activity, for example, immunoglobulins are protective proteins that are important in the transfer of passive immunity from the mother to the neonate which activates protective proteins that are important in the transfer of passive immunity from the mother to the neonate which activates the new-borns immune system and other metabolic processes. The natural defense mechanism of immunoglobulins are found in high concentrations in colostrum, the first milk, and low concentrations in milk thereafter. In addition to the immunoglobulins, other proteins found in milk are thought to have anti-microbial activities. The antibacterial effect of milk proteins is generally mediated by the reaction of hydrogen peroxide (H$_2$O$_2$), which is thought to be a major antibacterial substance. Lactoperoxidase, a known milk peroxidase, when combined together with H$_2$O$_2$ and iodide, produce a potent anti-bacterial system known as the Lactoperoxidase system.

The aims of the study
The first aim was to evaluate the level of H$_2$O$_2$ in mother’s milk in different postpartum periods. Many working mothers save their milk to be given later to their babies, but stored milk cannot be preserved in a hot climate at normal temperatures or in the refrigerator as this may allow fermentation. Therefore, the second aim was to evaluate the stability level of hydrogen peroxide during periods when frozen.

Methods and material
Methods
Sixty lactating mothers voluntarily donated their breast milk samples by manual expression or by using a breast pump. Ethical consent was obtained from the local area Ethical committee for all parts of the study. Each subject gave written consent after a full explanation of the procedures. The women were selected while attending a child-hood clinic with their neonate for routine medical examination - theirs ages were between (20-35) years, at various postpartum (PP) periods. The women were apparently healthy, non smokers and not taking any medication. Women with mastitis or other illness were excluded from the study. The samples were divided into four groups, 15 samples each. Milk samples were collected according to the time of (PP) lactating periods:

(i) From birth-7 days (first week collection, group-A)
(ii) From 8-14 days (second week collection group-B )
(iii) From 15-21 days (third week collection group-C)
(iv) From 22-30 days (forth week collection group-D)

The samples were placed in sterile plastic tubes and transported to the laboratory and kept on ice prior to analysis or kept in the freezer.

Each set of milk samples were collected in 20 mM EDTA solution. De-fatted milk samples were obtained by dividing whole milk samples into smaller portions and centrifuging them twice at alternate rates of 500g and 3000g at room temperature for 15 minutes, and collecting the aqueous phase of the milk each time by careful aspiration with a thin needle through the surface fat layer. During centrifugation, the milk separates into three layers, i.e. a cell pellet, a middle aqueous layer and an upper fat layer.

Key Words: hydrogen peroxide, lactation, breast feeding, mother’s milk, colostrum, Iraq

The aqueous layer was collected each time and processed immediately or stored at freezing point. Prior to testing for the level of H$_2$O$_2$ the frozen de-fatted milk samples were left for about 1 hour at room temperature.

**Materials and reagents**

We used a simple luminol-dependent chemiluminescence method to estimate the level of H$_2$O$_2$ in mothers milk at different stages after birth. The luminol dependent chemiluminescence is a sensitive indicator of free radicals and suitable for detection of traces of H$_2$O$_2$. The H$_2$O$_2$/OH – luminol system can be used to measure the total H$_2$O$_2$ capacity of the milk.

Luminol reagent was obtained from Sigma chemical company. A few drops of NaOH were added to luminol stock to give a solution of pH=9.8. The ultra sensitive photon counting system was designed and built in the Department of Physiology, University of Basrah, College of Medicine and used to determine single photons of light emitted in the course of a chemical reaction of the oxidation of luminol. The maximum spectral range of the 13 stages EMI photomultiplier tube used in the photon counting instrument is between 0.2 and 0.4 microns. The peak quantum efficiency of the EMI tube at 4000Å is 35%. The light emission was initiated by injection of 1ml of luminol-NaOH stock solution (pH9.8) into a cuvette vessel located in front of the photomultiplier tube containing 100µl of de-fatted milk sample or H$_2$O$_2$ at different concentrations in µM. All measurements were made in duplicate and the mean used to estimate H$_2$O2 concentration for each sample. The ultra sensitive photon counting system was designed and built in the Department of Physiology, University of Basrah, College of Medicine and used to determine single photons of light emitted in the course of a chemical reaction of the oxidation of luminol.

The maximum spectral range of the 13 stages EMI photomultiplier tube used in the photon counting instrument is between 0.2 and 0.4 microns. The peak quantum efficiency of the EMI tube at 4000Å is 35%.

**Results**

Standard H$_2$O$_2$ curves were obtained by injection of 1ml of Luminol stock solution to 100µl of H$_2$O$_2$ at different concentrations in µM. Chemiluminescence peak heights in mm were detected using chart recorder. A standard curve was drawn between peak heights (mm) against H$_2$O$_2$ concentration in µM to access the level of H$_2$O$_2$ in mother’s milk at different time of PP lactating periods. Milk samples were stored at freezing point for different periods of time and gave nearly same levels of H$_2$O$_2$ for up to four weeks storage (Table 1).

One way ANOVA statistical analysis was used to evaluate significance between different groups and paired t-tests were used to identify significant levels of H$_2$O$_2$ at different storage times at freezing point. After four weeks of storage at freezing temperature periods there were no significant differences (P>0.05) for any group.

**Table 1.** The concentration levels of H$_2$O$_2$ (µM) in milk samples as a function of time (weeks) of post-partum (PP) periods and at several weeks of storage at freezing temperature.

<table>
<thead>
<tr>
<th>Sample after PP periods (week)</th>
<th>H$_2$O$_2$ concentration (µM)</th>
<th>Fresh Milk Control collection</th>
<th>One week storage</th>
<th>Two week storage</th>
<th>Four week storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>First week collection</td>
<td>24.992 ± 0.168</td>
<td>24.467 ± 0.243</td>
<td>24.149 ± 0.184</td>
<td>23.729 ± 0.197</td>
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<td>Group A</td>
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<tr>
<td>Second week collection</td>
<td>20.400 ± 0.169</td>
<td>19.440 ± 0.925</td>
<td>18.361 ± 0.105</td>
<td>18.383 ± 0.276</td>
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<tr>
<td>Group B</td>
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<tr>
<td>Third week collection</td>
<td>15.783 ± 0.782</td>
<td>14.483 ± 0.252</td>
<td>14.333 ± 0.227</td>
<td>14.456 ± 0.330</td>
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<tr>
<td>Group C</td>
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<tr>
<td>Fourth week collection</td>
<td>08.75 ± 0.270</td>
<td>08.373 ± 0.274</td>
<td>08.033 ± 0.281</td>
<td>07.980 ± 0.300</td>
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<tr>
<td>Group D</td>
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</table>

Significance was found between groups at different times of postpartum periods of extracting the milk samples from the mothers. H$_2$O$_2$ levels were significantly different between groups 1 and 2 (P<0.05) and between groups 1 and 3 (P<0.01). A significantly greater difference was found between groups 1 and 4 (P<0.001).

**Discussion**

Recently H$_2$O$_2$ has been found to safely and successfully treat a wide variety of human diseases using a minimum diluted therapeutic amount without harmful side effects. Bio-oxidative therapies involve administering small amounts of diluted hydrogen peroxide into the body for the prevention and treatment of diseases. Studies have not yet determined the exact amount of H$_2$O$_2$ present in human milk. Levels may also differ in women from different countries or different climates.

The amount of H$_2$O$_2$ present in mother’s milk was detected with different concentrations at different stages of lactation (Table 1). The highest concentration was detected during the first week of lactation (24.992 ± 0.168µM) which decreased slightly at the end of the second week (20.4 ± 0.169 µM). The level of H$_2$O$_2$ fell sharply after the third and fourth weeks of PP period (Table 1). Therefore, it is important for the newborn to be fed during the colostrum stage since the level of H$_2$O$_2$ decreases significantly after the first week of lactation (P<0.05). The first week is important for the newborn to be fed by the mother because the immune system is not yet developed. The level of active units of H$_2$O$_2$ decreases in a highly significant manner (P<0.001) after four weeks of lactation (08.75 ± 0.27 µM).
Many mothers do not know the potential benefit of the first milk. It is the duty of the community/health worker to clarify the necessity of colostrum for the newborn. In addition, our results indicated that storage of mother’s milk at freezing temperatures for about four weeks does not reduce the level of H$_2$O$_2$ significantly ($P>0.05$). This leads us to say that the stability of H$_2$O$_2$ levels remain nearly constant during storage at freezing temperatures. This may suit many working mothers who are not able to feed their newborns immediately but can express their milk and store it at cold temperatures for later use.

In conclusion, mother’s milk contained a considerable amount of hydrogen peroxide which enhances the immature immunologic system of the newborn and strengthens its deficient host defense mechanisms against infective or other foreign agents. Since there were no significant differences in the levels of H$_2$O$_2$ during freezing of milk for one month, it can be stored for later use.

References

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Mother’s milk and hydrogen peroxide
母乳和过氧化氢


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测量产后期不同时段母乳中过氧化氢水平和冷冻储藏不同阶段的水平。使用发光氨过氧化氢依赖化学发光在 pH = 9.8 的技术。产后期的第一周发现过氧化氢水平最大值(24.992 ± 0.168 µM)。第二周显
著(\(P < 0.05\))降低为(20.4 ± 0.169 µM)，第三周又显著(\(P < 0.01\))降低为(15.783 ± 0.782µM)。第四周急剧降低为(8.75 ± 0.27 µM)，与第一周显著差别(\(P < 0.001\))。所有组冷冻储藏的过氧化氢的稳定水平至少1个月持续不变(\(P>0.05\))。

关键词：过氧化氢，哺乳期，乳房喂养，母乳，初乳，伊拉克