Original Article

Mother's milk and hydrogen peroxide

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H_2O_2 levels in mother's milk were measured at different times of postpartum periods after birth as well as after different times of storage at freezing temperature. The luminol H_2O_2 dependent chemiluminesence at pH = 9.8 technique was used. Maximum levels of H_2O_2 were found in the first week of the postpartum period (24.992 ± 0.168 µM). The levels of H_2O_2 decreased significantly (P<0.05) in the second week (20.4 ± 0.169 µM), while a significant (P<0.01) decrease in the level of H_2O_2 occured in the third week after birth (15.783 ± 0.782) µM. The levels of H_2O_2 fell sharply in the fourth week of lactation (8.75 ± 0.27 µM) with a significant difference relative to the first week (P <0.001). The stability of the H_2O_2 levels remained constant, at least for a period of one month, with storage at freezing point for all groups (P>0.05).

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

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.[1,2] 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 the new-borns immune system and other metabolic processes.[3-5] The natural defense mechanism of immunoglobulins are found in high concentrations in colostrum, the first milk, and low concentrations in milk thereafter.[5] In addition to the immunoglobulins, other proteins found in milk are thought to have anti-microbial activities.[6,7] The antibacterial effect of milk proteins is generally mediated by the reaction of hydrogen peroxide (H_2O_2), which is thought to be a major antibacterial substance.[7] Lactoperoxidase, a known milk peroxidase, when combined together with H_2O_2 and iodide, produce a potent anti-bacterial system known as the Lactoperoxidase system.[8-11]

The aims of the study

The first aim was to evaluate the level of H_2O_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 childhood 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.

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The aqueous layer was collected each time and processed immediately or stored at freezing point. Prior to testing for the level of H_2O_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 chemiluminesence method to estimate the level of H_2O_2 in mothers milk at different stages after birth.[12-14] The luminol dependent chemiluminesence is a sensitive indicator of free radicals and suitable for detection of traces of H_2O_2. The H_2O_2/ OH – luminol system can be used to measure the total H_2O_2 capacity of the milk.[12-15]

Luminol reagent was obtained from Sigma chemical company. All other reagents were purchased from various sources. Luminol was dissolved in dimethyisulphoxide (DMSO) to give a concentration of 0.01 Molar as a stock solution. Since luminol works optimally at alkaline pH a few drops of NaOH were added to luminol stock to give a solution of pH=9.8.[12-15]

The light emission was initiated by injection of one ml 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_2O_2 at different concentrations in µM. The cuvettes were protected from direct light in light-tight surroundings; measurements were conducted by an ultra sensitive photon counting system and recorded as a peak height in (mm) using a chart recorder.[16] The present chemiluminesence method was sensitive to a minute volumes (µl) at very low concentration of H_2O_2 (0.3ml of 3% H_2O_2, 1:10000 dilution). All measurements were made in duplicate and the mean used to estimate H2O2 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.[16] 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%.[16]

Results

Standard H_2O_2 curves were obtained by injection of 1ml of Luminol stock solution to 100 µl of H_2O_2 at different concentrations in µM. Chemiluminesence peak heights in mm were detected using chart recorder. A standard curve was drawn between peak heights (mm) against H_2O_2 concentration in µM to access the level of H2O2 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_2O_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_2O_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_2O_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.

		H_2O_2 concentration (µM)		
Sample after PP periods (week)	Fresh Milk Control	One week storage	Two week storage	Four week storage
First week collection *Group A*	24.992 ± 0.168	24.467 ± 0.243	24.149 ± 0.184	23.729 ± 0.197
Second week collection *Group B*	20.400 ±0.169	19.440 ±0.925	18.361 ±0.105	18.383 ±0.276
Third week collection *Group C*	15.783 ±0.782	14.483 ±0.252	14.333 ±0.227	14.456 ±0.330
Fourth week collection *Group D*	08.75 ±0.270	08.373 ±0.274	08.033 ±0.281	07.980 ±0.300

Significance was found between groups at different times of postpartum periods of extracting the milk samples from the mothers. H_2O_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_2O_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.[18] Bio-oxidative therapies involve administering small amounts of diluted hydrogen peroxide into the body for the prevention and treatment of diseases.[18-22]

Mother's milk contains a considerable amount of H_2O_2, especially during the colostrum period, which activates the new borns immune system and many other key metabolic processes.[17] Studies have not yet determined the exact amount of H_2O_2 present in human milk. Levels may also differ in women from different countries or different climates.

The amount of H_2O_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_2O_2 fell sharply after the third and forth weeks of PP period (Table 1). Therefore, it is important for the new born to be fed during the colostrum stage since the level of H_2O_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_2O_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 new born. 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_2O_2 significantly (P>0.05). This leads us to say that the stability of H_2O_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_2O_2 during freezing of milk for one month, it can be stored for later use.

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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