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Original Article

Estimation of vitamin C concentration and pH value in human Colostrum milk of quetta, City.

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Abstract
The vitamin C content of human Colostrum milk was determined among ten newly delivered mothers. Milk samples (colostrum) were obtained from Quetta city. The pH of the sample was found in the range of 6.1 -7.4 and the vitamin C were found in the range 2.9 – 5 per 100ml. Therefore results show that the required concentration of vitamin C is sufficient for the new born. But the higher value of pH and ascorbic acid increases the risk of breast cancer in mothers.

Key words: Human/Mammalian milk, Quetta, ascorbic acid, pH value.

1. Introduction
In this investigation different methods have been used to determine the concentration of ascorbic acid contents in human Colostrum milk. Also measured was the pH value of milk. The current study is carried out to measure the concentration of ascorbic acid and pH value of human milk which is necessary in many aspects. Colostrum (also known colloquially as beestings bisnings or first milk [1,2]) is a form of milk produced by the mammary glands of mammals immediately after pregnancy. Human body needs vitamin C because they lack the enzyme L-gulonolactone oxidase. Which catalyzes the final step in the conversion of glucose into ascorbate [18]. Human milk, is the milk produced by the breasts (or mammary glands) of women after the delivery of the new born [12]. Milk is the primary source of nutrition for newborns before they are able to eat and digest other foods. Human milk enhances the immature immunologic system of the neonate and strengthens host defense mechanisms against infective and other foreign agents [6]. In Pakistan, 72 infants die out of 1000 live births every year. But out of these 72 deaths, 53 are those who die before reaching the age of one month due to diarrhoea, pneumonia, respiratory infections and malnutrition [5, 22]. A major source of these diseases is lack of exclusive breastfeeding, use of unhygienic bottles, formula milk and teats. These infants can be saved if they are protected through natural protection of mother’s milk without use of any other food item like gutti (the first sweet liquid given to the baby by mouth on birth), honey or water for the first six months, they said. Lack of breastfeeding causes various diseases in infants including diarrhoea, pneumonia [5] W.H.O recommends exclusive breastfeeding for the first 6 months of life [3] Drugs are transferred from blood plasma across ductal cells to the milk by diffusion or active transport. The latter may result in higher concentration of the drug in the breast milk than in the plasma of the mother [4]. Drug characteristics that increase excretion in milk include. Weakly alkaline rather than weak acid.

1.1 Location
Quetta is the capital of Balochistan province in Pakistan. The total area of Quetta is 1,024 sq m approximately it is located at the west edge of Pakistan and located in high altitude i.e 5,260 ft above from sea level. Quetta district is consider the urban district of balochistan at 30°10′N 67°00′E coordinates. Different types of tribes are living in city the city is actually dominated by Pashtuns, with a very large number of Hazara community. Balochis and Punjabis form smaller minority groups. Other groups found in the city include Brahuis, and Sindhis. Large communities of Persian, Afghan Pashtun and Uzbek refugees are also found in the city, while the city is overwhelmingly with Muslim.

2. Sample collection
To determine the concentration of vitamin C and pH in human Colostrum milk we collected 10 samples from healthy not barren women, the ages ranged from 25 to 38 years with a median age of 29 one day delivered mothers from the right and left breasts of the same mother from...
different hospitals of Quetta city. During the month of August 2011 in a glass capped bottle and kept on dark place [7].

3. Method and material

3.1 Lowry, Lopez and Bressey method

Reagents: Trichloro acetic acid, sulphuric acid, 12 molar and 2,4-dinitrophenyl hydrazine, thiourea 5% solution, cupric sulphate 0.6% solution.

For determination of ascorbic acid by 2,4-dinitrophenyl hydrazine. A slight modification was done as described by Nino and Shah [8]. Chemical are used for analysis are buy from (BDH Ltd, England and E. merck F.R Germany). The ascorbic acid was converted to dehydro ascorbic acid by shaking with cupric sulphate solution and then coupled with 2,4-dinitrophenyl hydrazine in the presence of thiourea as a mild reducing agent. Sulphuric acid then converted dinitrophenyl hydrazine into a red colored compound, which was assayed calorimetrically. The thiourea is added to prevent oxidation of dinitrophenyl hydrazine reagent by interfering substance.

The vitamin C content in human milk was determined by the method developed by Lowry, Lopez & Bressey [9]. Milk samples were centrifuged at 3000 rpm for 20 mins. On centrifuge, the fat component of milk separates from the fluid part and moves upwards leaving a clear mid-zone, while the cells settle at the bottom. From the clear mid-zone, 0.3 ml sample was taken in a test tube to which 1.2 ml TCA was added (trichloro acetic acid) and mixed well. The mixture was then centrifuged at 3000 rpm for 10 mins. From this, 0.9 ml supernatant was taken and 0.4 ml DTC (2,4 dinitrophenyl hydrazine thiourea copper sulphate) was added. It was then covered with aluminium foil and incubated at 60°C for 60 mins in a water bath Immediately. After incubation, the sample was chilled in ice-cold water and 1.6 ml of 65% sulphuric acid was added gradually. Finally the treated sample was stored at room temperature for 30 mins. Absorbance was measured against a reagent blank at 520 nm by a spectrophotometer. Every sample was analyzed in triplicate.

3.2 pH Determination

The pH of human milk is immediately determined after collection with jenny way 3510 pH meter using glass electrode.

3.3 Statistical analysis:

Data obtained from above mentioned parameter was subjected to statistical analysis through Statistical package for the social sciences (PC software S.P.S.S Version 14).

3.4 Chemical structure and analysis

All chemicals modeling and analysis is carried out with the help of Chem Office Ultra 2002 cs3DChemDraw (Chemical information sciences p.c software pro Version 7.0).

4. Result and discussion

Vitamin C present in human milk has a number of biochemical functions linked to the function of the immune system. It helps in the maintenance of a natural barrier against infection, stimulates leukocytes for their phagocytic activity and anti-microbial activity, augments antibody production and complements levels [10] and also enhances synthesis of interferon [11]. For growth, development and survival, infants need an optimum supply of vitamin C. The human milk has a sufficient value of vitamin C but less amount of iron for infant [24] which fulfills the requirement of newborn. The vitamin C can help in the absorption of iron by converting [17] the inorganic Fe++ to Fe+++ . In 2008, breast cancer caused 458,503 deaths worldwide 13.7% of cancer deaths in women [13]. 90,000 breast cancer cases annually in Pakistani women. He said only 10 percent of women were diagnosed and that out of them about 75 percent women did not treat the problem and died within five years. Due to breast feeding there is a less risk of breast cancer [14]. The R.D.A of vitamin C for newborn (up to 6 month) is 35mg/day [19]. The Asian mother produce daily 780-1000ml (average) milk per day which is sufficient amount of vitamin C for infant [20].

Table 1. Show the concentration of vit C, pH value of human milk.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>pH of milk</th>
<th>Vitamin C concentration per 100ml (mean of three readings)</th>
<th>Result Age of mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.4</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>3.9</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>7.4</td>
<td>3.9</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>7.0</td>
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<td>37</td>
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<td>5</td>
<td>6.9</td>
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<td>31</td>
</tr>
<tr>
<td>6</td>
<td>6.9</td>
<td>3.3</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>7.2</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>7.1</td>
<td>3.8</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>6.1</td>
<td>2.9</td>
<td>38</td>
</tr>
<tr>
<td>10</td>
<td>7.0</td>
<td>3.7</td>
<td>25</td>
</tr>
</tbody>
</table>

Fig. 1 Show the structure of vitamin C

5. Hypothesis

The higher value of vitamin C (Ascorbic acid) and higher value of pH in milk shows that in alkaline milieu epithelial cells in breast are damaged as a result inside the breast the cell undergoes hyperplasia, marked cell atypai and marked increased the mitotic activity [15] which at least cause cancer by the same mechanism [16] in breast if mammary
gland secretions are not utilized. A cancer-causing mechanism of hexavalent chromium may be triggered by vitamin C [23]. The hypothesis of current study also provide the ground work for further investigation.

Acknowledgement
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