Original Article

# VITAMIN-A (BREAST MILK) ASSESSMENT BETWEEN RURAL AND URBAN AREA OF BALOCHISTAN BY H.P.L.C.

Muhammad Aziz [*], Saima Jadoon[*] ,Shafia parveen[**]
[*]Department of Chemistry University of Balochistan, Quetta Pakistan.
[**] F.G.E.I Institutions (C/G) Quetta.

**ABSTRACT**

The vitamin A (retinol) content in human milk was determined among 30 mothers.15 belonged to urban area and 15 belonged to rural area of Balochistan. Milk samples were obtained from Quetta urban and Mastung rural Districts to show the nutrition difference present between urban and rural population.

**Materials and methods:**

A rapid sensitive H.P.L.C method is used for separation and quantification of retinol in breast milk. The column used was Bonda pak $C_{18}$ .The mobile phase $CH_3OH$:$H_2O$ and the wavelength 325nm provided an excellent separation of this compound without interfering peak.

**Result:**

The vitamin A were found in the range 0.60-2.4ug/L in Quetta and 0.21-1.0ug/L in mastung Lactating women in rural part of Balochistan have lower milk vitamin A concentrations than lactating women in urban part of Balochistan.

**Conclusion:**

Results also show that the required concentration of vitamin A is not sufficient for the new born in mother milk and it also decreases with the passage of time in human milk.

**Key words**: Human/Mammalian milk, Quetta, Retinol, Chromatography.

## INTRODUCTION

Background: Few studies have investigated to show the concentration of Vitamin A in human milk by using different analytical methods[1],[3]. Others also measure food status and lifestyle factors between urban and rural populations in country[2].

The nutritional status of a population can be determined in various ways. Growth assessment is the best single measure to define the health and nutritional status of children. The current study is carried out to measure the concentration of Vitamin A in human milk which is necessary in many aspects. Night blindness or Xerophthalmia is primarily and basically caused by vitamin A deficiency[11]. The disease is also termed as night blindness and corneal abnormalities, in which softening of cornea and ulceration lead to irreversible blindness. Vitamin A deficiency is estimated to affect approximately one third of children under the age of five around the world[5]. But how much Pakistani children of Balochistan area are suffering from the deficiency of vitamin A, which become the cause of child mortality[12,13] is still a burning question.

Human body needs vitamin A because it has strong effect on the immunity of the body[8]. Milk produced by the mammary glands of mammals immediately after pregnancy[14]. Human milk enhances the immature immunologic system of the neonate and strengthens host defense mechanisms against infective and other foreign agents[6]. Drugs and vitamins are transferred from blood plasma across ductal cells to the milk by diffusion or active transport. The latter may result in

*Corresponding Author:*
*Muhammad Aziz*
*Department of Chemistry University of Balochistan, Quetta Pakistan.*
*Email.Aziz1sh@Hotmail.com*
*Tel +923368466246*

higher concentration of the drug in the breast milk than in the plasma of the mother[4].

**Location:**

Mastung District is consider as a rural district located in the northwest of Balochistan at Coordinates 29°45'N 67°00'E. On the other hand Quetta district is considered the urban district also located in northwest of Balochistan at 30°10'N 67°00'E coordinates. Distance between Quetta and Mastung is 53 km[9]. In Balochistan, there is a significant difference between urban and rural populations in terms of monthly income, daily workout, lifestyle and diet intake. Such differences affect the health and nutritional status of mother and infant which directly depend upon mother to fulfill diet requirement. The present study was, therefore, undertaken in order to assess the nutritional status of vitamin A as an indicator of the fat soluble vitamin in human milk in rural and urban areas of Quetta and Mastung District, Pakistan.

**Sample collection:**

To determine the concentration of vitamin A in human milk we collected 30 samples from healthy women.15 belonged to mastung and 15 belonged to quetta with the median age of child 13.00 months and 11.0 months respectively. We collected milk from the right and left breasts of the same mother from different hospitals of the city. During the month of august 2012 in a glass caped bottle and kept on dark place[7].

## METHOD AND MATERIAL

**Reagents:**

Ethanol, potassium hydroxide , n-Hexane ,Methanol and Pyrogallol .

Retinol in breast milk is found mostly as estrified fatty acid.

The ester linkage must be saponified to release the retinol. saponified with 1.5 ml 50% potassium hydroxide and ethanol (3:5).The mixture was vigorously vortexed and incubated at 37 °C for 2 hour under nitrogen. After incubation sample were mixed with 1ml n-hexane and centrifuge for 10 min @ 2000 rpm[10]. Retinol is extracted into n-hexane twice .The hexane layer is transferred to clean vial evaporated under nitrogen .Reconstitute in mobile phase and injected in the HPLC column separating by reverse phase HPLC using $C_{18}$ column and measured at 325 nm wavelengths.

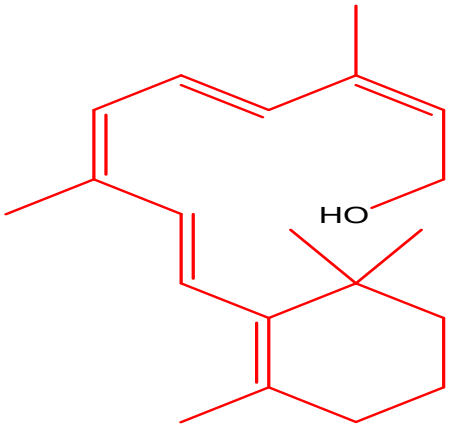
**Chemical structure and analysis:**

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)

**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).

**Fig 1 show the structure of Retinol**




retinol

C20H30O
Mol. Wt.: 286.45

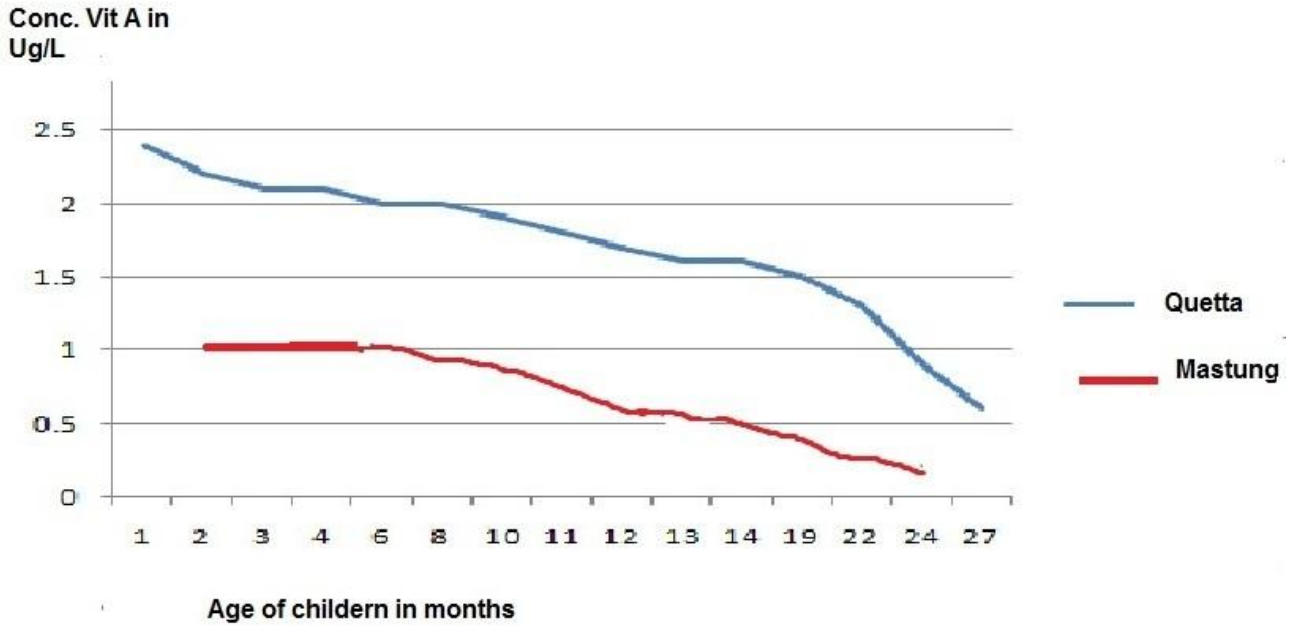
## RESULT AND DISCUSSION

**Table 1**

| S.No | Age of Child in months | Sex of Child | Concentration of Retinol in |
|---|---|---|---|
| 1 | 1 | M | 2.4 |
| 2 | 2 | F | 2.2 |
| 3 | 3 | F | 2.1 |
| 4 | 4 | M | 2.1 |
| 5 | 6 | F | 2.0 |
| 6 | 8 | M | 2.0 |
| 7 | 10 | F | 1.9 |
| 8 | 11 | M | 1.8 |
| 9 | 12 | F | 1.7 |
| 10 | 13 | F | 1.6 |
| 11 | 14 | M | 1.6 |
| 12 | 19 | F | 1.5 |
| 13 | 22 | M | 1.3 |
| 14 | 24 | F | 0.9 |
| 15 | 27 | M | 0.6 |

**Retinol (Vitamin A) concentration result from Quetta district.**

**Fig 2. Show decline in vitamin A Concentration during lactation months**.




On x- axis conc of vitamin A in ug/L ,Y-axis show the age of children in months

**Table 2.**

**Retinol (vitamin A) concentration result from Mastung district.**

| S.No | Age of Child in months | Sex of Child | Concentration of Retinol in ug/L |
|---|---|---|---|
| 1 | 3 | M | 1 |
| 2 | 6 | F | 1 |
| 3 | 7 | M | 0.99 |
| 4 | 9 | M | 0.98 |
| 5 | 10 | F | 0.96 |
| 6 | 11 | M | 0.93 |
| 7 | 12 | F | 0.89 |
| 8 | 13 | F | 0.75 |
| 9 | 14 | M | 0.68 |
| 10 | 15 | M | 0.59 |
| 11 | 17 | M | 0.45 |
| 12 | 18 | M | 0.39 |
| 13 | 19 | F | 0.30 |
| 14 | 22 | M | 0.24 |
| 15 | 24 | M | 0.21 |

**Table 3 One-Sample Statistics**

| Name | No of obs | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| **Quetta** | 15 | 1.7133 | .48970 | .12644 |
| **Mastung** | 15 | .6907 | .30274 | .07817 |

| Name | Test Value = 400 | | | | | |
|---|---|---|---|---|---|---|
| | t | df | Sig. (2-tailed) | Mean Difference | 95% Confidence Interval of the Difference | |
| | | | | | Lower | Upper |
| **Quetta** | -3149.983 | 14 | .000 | -398.2867 | -398.5579 | -398.0155 |
| **Mastung** | -5108.460 | 14 | .000 | -399.3093 | -399.4770 | -399.1417 |

**Single T-test:**

T-test is use for the significance of the difference between the observed mean of a sample and a hypothetical mean of the population from which the sample is randomly drawn. Where 400 ug/L is vitamin A RDA value for children

The proposal to use Vitamin A concentration in human breast milk, as an indicator of vitamin status of a Quetta district and Mastung district is relatively new area. Retinol in human breast milk was highly protein bound organic compound. Mostly in breast milk esterified to fatty acid. The ester linkage must be saponified to release retinol.

**Comparison between urban and rural participants:**

Table 1 shows the value of retinol in human breast milk from Quetta district. Here much more facilities available as compare to Mastung area. In Quetta it also has come to

know that mothers are healthy and aware about their diet requirement during lactating period.

Table 2 shows the more deficiency of vitamin A in human milk of Mastung area mothers and they are not aware about diet requirement during lactating period .In Mastung district mothers are weak and pale in complexion due to deficiency of Vitamin A and other malnutrition in diet. It has also come to our knowledge that with the increase in the child age the concentration of vitamin A in milk will decrease as shown in fig 2.Milk is the primary source of nutrition for newborns before they are able to eat and digest other foods The increase in the practice of breast-feeding, associated with the slogan "breast feed is best" and that breast milk does not require any other supplementation and fulfills the RDA value of infant .If the infant only drinks breast milk, he ultimately may lead to deficiency of vitamin A and become victim of night blindness.

## CONCLUSION

In this review, we are able to say that the reason of vitamin A deficiency shown in table 4 in infants is due to breast-feeding only and Lactating women in rural part of Balochistan have lower milk vitamin A concentrations than lactating women in urban part of Balochistan. It has been observed that Fat soluble components in milk other than triglycerides decline during early lactation but not the same extent.

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