EXPOSURE TO OESTROGENS BY INDIAN WOMEN, A SCARE OF ONCOGENECITY

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ABSTRACT

Objective: To measure the oestrogen concentrations in various supply of milk and milk products in Pune.

Background: Indian women consume milk every day. Breast and ovarian cancer are on rise in India. Oestrogen has been reported to be strongly associated with increased risk for breast cancer. The daily intake of milk could be a risk factor because of various attempts of hormone injections to cow/buffalo for increasing the milk production.

Methods: Milk and milk products being consumed in Pune were measured for oestrogen concentration using Brown's method for extraction and CLIA for measurement.

Results: The median oestrogen concentration in any milk /milk product was 180 pg/gm which is far below the oncogenecity level of 320 pg/mL.

Conclusion: The daily consumption of dairy products is advocated to be not harmful because of its non-significant levels of oestrogen, on the contrary, a beneficial factor from health point of view in vegetarian Indian women as a supply of micronutrients scores out as safer.

Keywords: Oestrogen; Aetiology; Oncogencity

INTRODUCTION

Epidemiological and experimental studies have shown the role of oestrogens in the aetiology of breast cancer in humans probably acting through hormone-related pathways (1). Increased concentrations of circulating oestrogens have been found to be strongly associated with increased risk for breast cancer in postmenopausal women (2). It has also been hypothesized that oestrogen exposure is a major determinant of risk for breast cancer. Animal studies have shown that oestrogens promote mammary tumours and oophorectomy or anti-oestrogenic drug reverse the effect (3). Exposure to exogenous oestrogens from a variety of sources has become increasingly common, particularly from hormonal preparations for use as contraceptives or to cure the symptoms of the menopause (4). Hormone replacement therapy in older women containing oestrogens has become common (5). It has also been thought to be a risk factor for various clinical problems like obesity, pubertal disorders and various kinds of cancer like breast cancer and prostate cancer. Estrogen has been shown to reduce the serum levels of gonadotrophins in pre-pubertal children and affect ovulation in adult females (2). One potential dietary source is milk. Absolute quantities of total oestrogen was measured in a variety

of commercial milks (whole, 2%, skim, and buttermilk) and found that the milk products contain considerable levels of oestrogen. The levels in skim milk were lower than that observed in whole milk, 2% milk, and buttermilk. Butter milk contained the lowest, while curd contained the highest, probably because estrogen is fat soluble hormone (1). Ganmaa et al. (4) pointed out that milk and dairy products represent the main source for animal estrogens adding up to 60–70% of all animal estrogens consumed. Modern genetically improved dairy cows continue to lactate throughout almost the entire pregnancy. Therefore, cow's milk contains large amounts of estrogens. So far women do not have any information on the estrogen content of the milk being consumed. Therefore, we examined concentrations of oestrogen in the various sources of milk and milk products being supplied in Pune city.

MATERIALS AND METHODS

Estimation of Estrogen in milk and milk products was carried out by Brown's method (1955), modified in the laboratory (6). It was estimated by the following protocol:

The protocol was divided in two methods –

1. Manual extraction method
2. Instrumental analysis

1. Manual extraction method:

10 ml / gm of milk / milk product was taken in a glass bumper tube. 2ml of Conc. Hydrochloric Acid (HCl) (name of the company) was added in that and this mixture was mixed on vortex. The tube was then kept in boiling water for about 45 min – 1 Hr., which after removing was cooled at room temperature for about 10-15 min. and then cooled completely in ice. The hydrolysate was extracted with 40 ml Diethyl ether (Fischer Scientific) by keeping it on magnetic stirrer for about 15 min. The aqueous phase was discarded and the ether layer was extracted with 5-6 ml of 1 N Sodium hydroxide (NaOH) (name of the company). Lower alkaline layer was collected in which 800 mg Sodium Bicarbonate ($NaHCO_3$) (name of the company) was added and mixed thoroughly. This alkaline / neutralized solution was extracted with 20-25 ml diethyl ether (Fischer Scientific) by keeping it on magnetic stirrer for about 5 min. Organic ether layer was collected in a test tube and was evaporated to complete dryness. This was dissolved in measured assay buffer / diluent.

2. Instrumental analysis:

The estrogen content was estimated using CLIA technique on Architect (Abbott) Immunoassay analyzer.

The final estrogen value in the given sample was calculated per 100 ml / per 100 gm.

3. CLIA Technique:

Antibody coated micro-particles were reacted with the test sample. The Antibody bound estrogen reacts with fluorescence tagged estradiol antibody and the bound fraction having fluorescence was measured. Instrument was calibrated using internationally accepted calibrators and internal quality controls were used for quality assessment. The inter and intra assay variation was 5.3% and 3.4 % respectively

For all the samples, the milk and milk products were processed in such a way to replicate Indian scenario. Milk was boiled and cooled and then used without cream for sample processing.

All the milk cream was used for making homemade curds in which 1 gm of commercially available Govardhan curd was used as an inoculum. For all the homemade butter milk samples, water was added in all the homemade curds (curd:

water -1:10 w/v) to make butter milk and mixed in a mixer. All the butter was removed and only lower butter milk was used for sample processing.

RESULTS

Milk from different sources of supply in Pune does not cross the limit of toxic level. Table 1 shows the oestrogen concentrations in various sources of milk supply in Pune. Milk oestrogen contents were below 320 pg/mL. Only the curds prepared from milk exhibited 424 and 400 pg/mL (Table 1 and 2). Krishnai milk contained 256 pg/mL and curd prepared from Amul and Chitale milk contained 424 pg/gm and 340 pg/gm respectively. However, the women consume not more than 150 mL every day. Commercially available milk powders also showed less than 320 pg/gm of oestrogen concentrations (Table 3).

DISCUSSION

Our results indicate that the milk supply in Pune contains about 180 pg/gm of estrogen in terms of any dairy product that is being consumed is far away from the level of oncogenicity. Curd is found to have maximum concentration (424 pg/gm) and the amount consumed is not more than 200 gm per day. Hence, milk or milk product intake may not be a causative factor for cancer (breast cancer) in Indian scenario.

Maruyama et al (1) reported that estrogens in milk were absorbed, and gonadotropin secretion was suppressed, followed by a decrease in testosterone secretion in male adults and children. Sexual maturation of prepubertal children could be affected by the ordinary intake of cow milk.

Hjartåker et al. (5) carried out a prospective cohort study in almost 50,000 Norwegian women examining childhood milk consumption and breast cancer incidence in later adult life. Milk intake during adult life was negatively correlated with breast cancer incidence. Subjects drinking more than 3 glasses of milk each day had a 44% lower incidence rate than women not drinking milk. There was no clear-cut association between various milk preparations and milk fat consumption. Combination of the data on childhood and adult life milk consumption revealed a significant negative trend for the incidence rate with increasing amount of milk consumed showing a protective effect of milk consumption. Gallus et al. (14) reported a large integrated network of hospital-based case-control-studies from Italy. The authors detected an inverse association between milk intake and colorectal, breast and ovarian cancer and concluded that dairy products do not appear as strong risk predictors for the carcinomas.

Moorman and Terry (16-17) strongly focused on epidemiological data from cohort or case-control-studies. They opined that most trials were unable to produce a clear-cut pattern of increased or diminished breast cancer risk. Therefore, they concluded that the epidemiological literature available did not contain strong evidence for an association between milk consumption and later breast cancer risk. Calcium and vitamin D contents of dairy products probably reduce breast cancer risk. Hence, our results stating that the oestrogen concentrations in milk and milk products do not exceed the undesirable limits and claim that consumption is safe as far as oncogenicity are concerned.

Limitation of the study is that we have not measured estrogen concentration season-wise.

Table 1: Maximum estrogen concentration was 256 pg/mL in Krishnai milk and the curd prepared from Amul milk and Chitale milk were 424 and 340 pg/gm respectively.

Sr. No	Brand	Category	Sample Type	Boiled & Cooled	Reading on Architect Pg/ml	Diluent Used µl	Final Value Pg / 100 ml	Curd Sample	Reading on Architect Pg/ml	Diluent Used µl	Final Value Pg / 100 gm	Butter Milk	Reading on Architect Pg/ml	Diluent Used µl	Final Value Pg / 100 ml
1	Amul	-	Tonned Milk	No	51	200	102	-			-	-			-
2	Katraj	-	Tonned Milk	No	90	200	180	-			-	-			-
3a 3b 3c	Amul	Buffalo	milk(a)	Yes	31	400	124	Curd(b) made from Amul Buffalo milk	106	400	424	Butter Milk (c) Made from Curd of Amul Curd	<10	400	<40
4a 4b 4c	Chitale	Buffalo	milk(a)	Yes	51	400	204	Curd(b) made from Chitale Buffalo milk	85	400	340	Butter Milk (c) Made from Curd of Chitale Curd	<10	400	<40
5a 5b 5c	Gokul	Buffalo	milk(a)	Yes	27	400	108	Curd(b) made from Gokul Buffalo milk	23	400	92	Butter Milk (c) Made from Curd of Gokul Curd	21	400	84
6a 6b 6c	Shrirang	Buffalo	milk(a)	Yes	19	400	76	Curd(b) made from Shrirang Buffalo milk	55	200	110	Butter Milk (c) Made from Curd of Shrirang Curd	<10	200	<20
7	Sane	Buffalo	Milk	Yes	20	400	80								
8	Chitale	Cow	Milk	No	61	200	122	-			-	-			-
9a 9b	Pride of Cows	Cow	Milk (a)	No	64	200	128	-			-	Butter Milk (b) Made from Curd of Pride of Cows Milk	30	400	120
10	Pride of Cows	Cow	Milk	Yes	61	200	122	-			-	-			-
11a 11b 11c	Sane	Cow	Milk(a)	No	20	400	80	Curd (b) Made from Sane Milk	52	200	104	-			-
12a 12b 12c	Sphurti	Cow	Milk (a)	Yes	51	400	204	Curd (b) Made from Sphurti Cow Milk	63	400	252	Butter Milk (c) Made from Curd of Shurti Curd	19	400	76
13a 13b 13c	Krushnai	Cow	Milk (a)	Yes	64	400	256	Curd (b) made from Krushnai Cow Milk	80	400	320	Butter Milk (c) Made from Curd of Krushnai Curd	22	400	88
14	Chitalekar	Cow	milk	Yes	19	200	38								
15	Gavali (gaikwad)	Cow	milk	Yes	<10	200	<20								
16	Gavali (Javalkar)	Cow	milk	Yes	<10	400	<40								
17	Gavali (Javalkar)	Buffalo	milk	Yes	<10	400	<40								
18	Gavli (mokashi)	Cow	Milk	Yes	17	200	34	-			-	-			-
19	Gavali (Pebbles II)	Cow	Milk	Yes	38	400	152								
20	Gavali (Pebbles II)	Buffalo	Milk	Yes	48	400	192								

Table 2: None of the milk powder exhibited more than 100 pg/gm of oestrogen.

Sr. No	Brand	Category	Sample Type	Boiled & Cooled	Reading on Architect Pg/ml	Diluent Used µl	Final Value Pg / 100 ml	Curd Sample	Reading on Architect Pg/ml	Diluent Used µl	Final Value Pg / 100 gm	Butter Milk	Reading on Architect Pg/ml	Diluent Used µl	Final Value Pg / 100 ml
21a 21b	Govardhan	-	-	-			-	Commercially Available Curd (a)	90	200	180	Butter Milk (b) Made from Curd of Govardhan Curd	45	400	180
22	Amul	-										Commercially available spiced butter milk	<10	400	<40
23	Chitale	-						Commercially available Curd	28	400	112				
24	Amul Premium	-						Commercially available Premium Curd	21	400	84				
25	Amul	-										Commercially available non-spiced butter milk	31	400	124
26	Sphurti	-						Commercially available curd	46	400	184				
27	Katraj	-										Commercially available spiced butter milk	20	400	80
28	Shrirang	-						Commercially available curd	47	400	188				
29	Amulya*	Milk Powder	Milk made from Powder	No	13	400	52								
30	Nestle*	Milk Powder	Milk made from Powder	No	31	400	124								

Note: - * 1 gm of milk powder was dissolved in 10 ml of DW to make milk and then used for sample processing.

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Table 3. The median estrogen concentrations are less than 320 pg/mL in all the samples.

Oestrogen (pg/mL or pg/gm)		Milk	Curd	Butter milk	Milk Powder
Mean		113.1	190.4	106.0	88.0
Median		115.0	180.0	102.0	88.0
Std. Deviation		69.3	117.6	60.0	50.9
Minimum		20.00	84.00	40.00	52.00
Maximum		256.00	424.00	180.00	124.00
Percentiles	25	47.5	101.0	50.0	62.0
	75	173.0	264.0	166.0	132.5

CONCLUSIONS

Our data on milk and milk products indicate that estrogens in milk were below the so-called toxic levels. We also observed that the oestrogen concentrations in milk being consumed did not vary during the period. Therefore, consumption of milk does not lead to higher circulating oestrogen levels and is not responsible for oncogenicity. Milk for calcium and vitamin D contents need to be studied

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