Caries Protective Agents in Human Milk and Bovine Milk: An *in vitro* Study

Vabitha Shetty * / Amitha M Hegde ** / Nandan S *** / Suchetha Shetty ****

Objectives: *To estimate Calcium and Phosphorus withdrawal from hydroxyapatite in the presence of bovine milk and human milk from which the following protective fractions namely Casein, Whey protein, Lactose and Milk fat have been individually removed and to compare the above protective fractions in human and bovine milk.* ***Method***: *Human milk obtained from lactating mothers in the labor ward of Kshema hospital was subjected to immediate analysis. Bovine milk was obtained from a local dairy. Equal quantities of human milk and bovine milk (1ml) were separately subjected to the systematic removal of the four milk fractions. As each fraction was removed, the remaining milk samples were subjected to testing. Powdered hydroxyapatite from human dental enamel was subjected to demineralization with the addition of the milk sample under test for 20 minutes. This mixture was then centrifuged. Aliquots of the supernatant were taken for calcium and Phosphorus analysis using photospectrometry. Ten demineralization tests were similarly carried out for every milk fraction for both human and bovine milk separately. Equal samples of whole bovine milk and whole human milk were also subjected to similar testing.* ***Results***: *The calcium and phosphorus dissolution values were higher when the individual fractions were eliminated from both human milk/enamel samples and bovine milk/enamel samples as compared to the values obtained from whole human milk/whole bovine milk /enamel samples. Further, higher calcium and phosphorus dissolution values were observed when the fractions were individually and separately removed from the whole human milk/enamel samples as compared to the corresponding values obtained when these fractions were removed from bovine milk/enamel samples.* ***Conclusion***: *The evaluated milk fraction in bovine milk namely casein, whey protein, lactose and milk fat were individually more caries protective when compared to the corresponding fractions in human milk.*

Keywords: *Demineralization, caries protective fractions, human milk, bovine milk.*
J Clin Pediatr Dent 35(4): 389–392, 2011

INTRODUCTION

Milk is one of the natural products consumed by every human being. It is a complex colloidal mixture of proteins, fats, lactose, minerals and many other constituents, some in suspension while some in solution.[1]

Milk and milk products contain a variety of agents that can suppress caries progression and some which can exert active caries preventive effects. The major advantages of any protective agents found in milk or milk products are that it would be perceived as a natural product, also milk is recommended on account of its nutritional properties chiefly as a source of calcium and protein.[1]

Bovine milk is generally regarded as being protective against caries, mainly due to the high calcium and phosphorus, but also because of buffering activity of the milk protein.[2] Human milk contains 1-1.5% protein compared with approximately 3.3% in bovine milk. However qualitative differences exist in the fats of human milk and cow's milk which could also principally of the triglycerides olein, palmitin and stearin but human milk contains twice as much of the absorbable olein.[3]

Most published research on the anticariogenic properties of milk proteins concentrate on the whole casein, its hydrolysates such as casein phosphopeptides or some proteose peptone fractions. Whey proteins have also been studied for their role as functional ingredients as well as for their anticariogenic properties. It is possible that whey proteins as

* Vabitha Shetty, Professor, Department of Pedodontics and Preventive Children Dentistry, A.B. Shetty Memorial Institute of Dental Sciences.
** Amitha M Hegde, Professor and Head, Department of Pedodontics and Preventive Children Dentistry, A.B. Shetty Memorial Institute of Dental Sciences.
*** Nandan S , Post Graduate Student, Department of Pedodontics and Preventive Children Dentistry, A.B. Shetty Memorial Institute of Dental Sciences.
**** Suchetha Shetty, Professor, Department of Biochemistry, K.S. Hegde Medical Academy, Department of Pedodontics and Preventive Children Dentistry, A.B. Shetty Memorial Institute of Dental Sciences.

Send all correspondence to: Dr. Amitha M. Hegde, Professor and Head of the Department, Department of Pedodontics and Preventive Children Dentistry, A.B. Shetty Memorial Institute of Dental Sciences, Derlakatte, Mangalore-575018, Karnataka, India.

Fax : 0824-2204572

Email: amipedo@yahoo.co.in
nandudent@rediffmail.com

a whole or after hydrolysis, could exhibit some cariostatic effects that might find applications in food and other formulations. It has also been suggested that milk fat could also prevent dental caries, either through a physical or a microbiological mechanism.[4]

In the present day scenario, in most of the developing nations, it is observed that there is an increasing usage of bovine milk for infants and young children as working women constitute a substantial part of the work force. At the same time the prevalence of Early Childhood caries is increasing.[5]

Since there is a paucity of data regarding the comparison of caries protective agents in bovine milk and human milk, the caries protective agents namely casein, whey protein, lactose and milk fat in bovine milk and human milk were evaluated and compared.

MATERIALS AND METHOD

Human milk was collected after informed consent was obtained from lactating mothers admitted in the labour ward of Kshema hospital and subjected to immediate analysis. Bovine milk was collected from a local dairy and was used immediately. The milk in the dairies had been obtained from the native/local cows.

Equal quantities of collected human milk and bovine milk (1ml) were separately subjected to the systematic removal of four milk components, namely Casein, Whey proteins, Lactose and Milk fat respectively and was subjected to testing.

Fresh samples of human and bovine milk (1ml) were taken and subjected to fractionation procedures.[1]

Removal of casein

Casein was initially removed by the coagulation method. Milk (1ml) was diluted with equal volume of water. 10% Hydrochloric acid was added dropwise until coagulation was achieved. After centrifugation at 1500g for 5 minutes, the supernatant was separated and the Casein precipitate was separated.

Removal of major whey proteins

The major whey proteins, which are readily heat denatured were insolubilized and removed from the supernatant by the procedure described in the previous section, then subjected to boiling for 5 minutes, allowing it to cool, and decanting the supernatant from the precipitate.

Removal of lactose

Lactose was precipitated from the supernatant by the addition of 10 ml acetone. After standing for 5 minutes, the mixture was centrifuged at 1500g for 5 minutes. The clear liquid fraction was then evaporated under reduced pressure.

Separation of milk fat

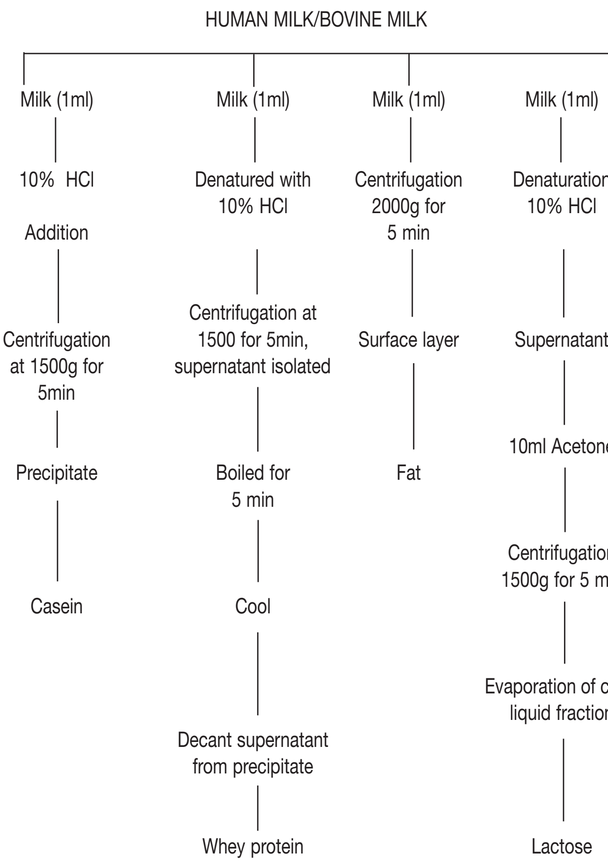
Milk was centrifuged at 2000g for 5 minutes, providing a surface layer of fat and fat associated materials and a clear aqueous lower layer.

Demineralization of hydroxyapatite and estimation of calcium and Phosphorus withdrawal

- 0.010g powdered hydroxyapatite from human dental enamel was subjected to the action of 10ml 0.2 M acetic acid at pH 4.2 with the addition of the milk sample under test for 20 minutes with shaking at 22ºC. This mixture was then centrifuged at 2000g for 10 minutes. Aliquots of the supernatant were taken for calcium and phosphorus analysis which was done using photospectometry.[1]
- Ten successive demineralization tests were similarly carried out for every milk fraction for both human & bovine milk separately.
- Equal samples of whole bovine milk and whole human milk were also subjected to similar tests.

Results were subjected to statistically analysis using students ‘t’ unpaired test.

FRACTIONATION PROCEDURES FOR BOVINE MILK AND HUMAN MILK



RESULTS

When the mean values of calcium and phosphorus dissolution from whole bovine milk–enamel samples (31.64mg/dl & 20.52mg/dl) and whole human milk–enamel samples (34.25mg/dl & 23.04mg/dl) following demineralization procedure were compared, no significant differences were found (Table 1).

It was found that mean values of calcium and phosphorus dissolution were higher when casein was removed from whole bovine milk-enamel samples (31.22mg/dl & 22.80mg/dl) and whole human milk-enamel samples (34.25mg/dl & 25.24mg/dl) when compared to the corresponding values obtained from whole bovine milk-enamel samples and whole human milk-enamel samples respectively which were statistically very highly significant (Table 1 and 2). Higher mean values of calcium and phosphorus dissolution were observed when whey protein, lactose and milk fat were individually and separately eliminated from whole bovine milk enamel samples (33.56mg/dl, 22.62mg/dl for whey protein, 35.23mg/dl, 23.70mg/dl for lactose and 33.89mg/dl, 23.33mg/dl for milk fat) when compared to the corresponding values obtained from whole bovine milk enamel samples following demineralization procedure (31.64mg/dl & 20.52mg/dl).

Similarly, significant differences in mean values of Calcium and Phosphorus dissolution were found when whey protein, lactose and milk fat were separately eliminated from whole human milk enamel samples (36.45mg/dl & 27.15mg/dl for whey protein, 36.90mg/dl & 25.64mg/dl for lactose and 39.05mg/dl & 32.31mg/dl for milk fat) as compared to the corresponding values obtained from whole human milk enamel samples (31.22mg/dl & 23.04mg/dl) (Table II). It was observed that the mean values of calcium and phosphorus dissolution were higher when casein was removed from the human milk–enamel samples (35.75mg/dl& 25.24mg/dl) when compared to the values obtained when casein was removed from bovine milk-enamel samples (34.25mg/dl& 22.80mg/dl) which was statistically very highly significant (Table 3).

Higher mean calcium and phosphorus dissolution values were also observed when the other fractions, whey protein, lactose and milk fat were individually and separately removed from human milk- enamel samples as compared to the values obtained when these fractions were removed from bovine milk-enamel samples (36.45mg/dl>33.56mg/dl & 27.15mg/dl>22.62mg/dl for whey protein, 36.90mg/dl> 35.23mg/dl & 25.64mg/dl>23.70mg/dl for lactose and 39.05mg/dl> 33.89mg/dl & 32.31mg/dl>22.33mg/dl for milk fat) Table 3.

DISCUSSSION

Milk is an excellent protein food that provides essential amino acids and organic nitrogen for humans and animals of all ages.[1] Milk also contains factors that have protective properties such as calcium, phosphate, casein and lipids. Research suggests that the benefits of milk and related products can be traced to several principal factors: reminerlization of the tooth, prevention of bacterial attachment to the tooth and inhibition of bacterial biofilm formation ability.[6]

Several recent studies from the United States and Europe have all associated milk or dairy product consumption with lower caries experience when combined with a normal rou-

Table 1. Comparison of Calcium and Phosphorus dissolution from whole bovine milk-enamel samples before and after withdrawal of the various milk fractions.

Milk samples	Calcium		Phosphorus		P-value
	Mean (mg/dl)	Std deviation	Mean (mg/dl)	Std deviation	
Whole bovine milk	31.64	4.9	20.52	3.94	<.0005
Whole bovine milk – Casein	34.25	2.3	22.80	2.64	
Whole bovine milk	31.64	4.9	20.52	3.94	<.0005
Whole bovine milk – Whey protein	33.56	4.1	22.62	2.47	
Whole bovine milk	31.64	4.9	20.52	3.94	<.0005
Whole bovine milk – Lactose	35.23	4.8	23.70	3.04	
Whole bovine milk	31.64	4.9	20.52	3.94	<.0005
Whole bovine milk – Milk fat	33.89	3.84	22.33	3.20	

Table 2. Comparison of Calcium and Phosphorus dissolution from whole human milk-enamel samples before and after withdrawal of the various milk fractions.

Milk samples	Calcium		Phosphorus		P-value
	Mean (mg/dl)	Std deviation	Mean (mg/dl)	Std deviation	
Whole human milk	31.22	1.36	23.04	1.20	<.0005
Whole human milk – Casein	35.75	2.41	25.24	1.64	
Whole human milk	31.22	1.36	23.04	1.20	<.0005
Whole human milk – Whey protein	36.45	2.08	27.15	1.76	
Whole human milk	31.22	1.36	23.04	1.20	<.0005
Whole human milk – Lactose	36.90	1.89	25.64	1.43	
Whole human milk	31.22	1.36	23.04	1.20	<.0005
Whole human milk – Milk fat	39.05	1.77	32.31	2.87	

Table 3. Comparison of Calcium and Phosphorus dissolution between bovine milk-enamel and human milk-enamel samples before and after fractionation procedures.

Milk samples	Calcium		Phosphorus		P-value
	Mean (mg/dl)	Std deviation	Mean (mg/dl)	Std deviation	
Whole bovine milk	31.64	4.90	20.52	3.9	0.79
Whole human milk	31.22	1.36	23.04	1.4	0.73
Whole bovine milk – Casein	34.25	2.45	22.80	1.25	<.0005
Whole human milk –Casein	35.75	2.60	25.24	1.6	
Whole bovine milk– Whey protein	33.56	2.15	22.62	2.08	<.0005
Whole human milk– Whey protein	36.45	2.58	27.15	2.40	
Whole bovine milk – Lactose	35.23	3.10	23.70	1.84	<.0005
Whole bovine milk – Lactose	36.90	3.29	25.64	2.14	
Whole bovine milk – Milk fat	33.89	3.28	22.33	2.34	<.0005
Whole bovine milk – Milk fat	39.05	3.79	32.31	2.87	

tine of oral hygiene.[6] Earlier studies of the milk fractions responsible for reduction of caries focused on casein phosphopeptides, but it was possible that other constituents of milk could also play a part.[1]

The results of present study have suggested that the caries protective actions of whole human milk were comparable to whole bovine milk (Table 3).These findings are at variance from the results obtained by Rugg Gunn *et al* who had conducted enamel dissolution experiments with bovine milk, human milk, lactose and sucrose solution. They observed increased amount of calcium dissolved in the human milk/enamel incubates demonstrating a decreased level of protection against enamel dissolution, as compared with bovine milk.[2] However the comparable caries protective actions of human milk and bovine milk in our study could be attributed to the fact that human milk samples were collected from new mothers and caries protective action could have been higher in milk obtained from such mothers.

One of the main findings of our study was that the removal of casein, whey protein, lactose and milk fat from both bovine and human milk resulted in an increased demineralization of hydroxyapatite which was statistically very highly significant (Table 1 and 2). This demonstrated the protective effect of the above factors. Reynolds et al found that the synthetic octopeptide calcium phosphate complex significantly reduced caries activity, confirming that this calcium phosphate stabilizing portion of the casein phosphopeptides is associated with anticariogenicity. The proposed mechanism of anticariogenicity for CPP-CP complexes is that they substantially increased the level of calcium phosphate in plaque, depressing enamel demineralization and enhancing remineralisation.[7]

The results of our study have revealed that casein exhibited a greater protective effect than whey protein in human milk while in bovine milk we observed greater protective effect exhibited by whey protein. E A Warner et al compared the anticariogenic effects of casein and whey protein fractions in vitro using a model system based on hydroxyapatite. The results of their study showed that although whey protein was less effective than CPP, they exhibited some protective effects and were therefore potentially useful anticariogenic agents.[4] Reynolds E C and Del Rio A have suggested that whey protein exert a topical anticariogenic effect by acting as a buffer.[7]

In our study, milk fat exhibited significant protective effect on hydroxyapatite dissolution in both bovine and human milk. It has been suggested that milk fat could also prevent dental caries, either through a physical or a microbiological mechanism; the former minimizes the adherence of the food to the tooth surface, while the latter involves the bacteriostatic properties of medium chain fatty acids.[1]

The data from the present study showed that all the four milk fractions namely casein, whey protein, lactose and milk fat in bovine milk had individually and separately a significantly greater protective effect on hydroxyapatite dissolution as compared to the respective fractions in human milk.

Our findings reveal that many fractions of bovine milk are more caries protective than their corresponding fractions in human milk. However this might suggest that bovine milk is superior to human milk.

Though our study concludes that bovine milk had higher caries protective properties in terms of these four protective factors, the immune factors present in human milk also may play a significant role in the caries protective mechanism. Human milk contains bacterial and viral antibodies, including relatively high concentration of secretory IgA antibodies, all of which play a vital role in the general and oral defense mechanism.[3]

Lysozyme is an effective antibacterial enzyme isolated from milk, tears and saliva. Human milk is a better source of lysozyme (2.5mg/100ml) than bovine milk (0.025mg/100ml).[8] Hence the benefits of breast feeding far outweigh any harmful effects which may very occasionally be observed. Simple preventive measures such as careful tooth cleaning from an early age and administering fluoride drops where the water supply has less than 0.7 ppm of fluoride should do much to prevent the onset and development of early childhood caries resulting either from breast milk or bovine milk.

CONCLUSIONS

1. Caries protective potential of whole bovine and whole human milk are comparable.
2. The four milk fractions in bovine and human milk namely casein, whey protein, lactose and milk fat individually exhibited significant inhibitory action on hydroxyapatite demineralization.
3. The evaluated milk fractions namely casein, whey protein, lactose and milk fat were individually more caries protective in bovine milk when compared to the corresponding fractions in human milk
4. The order of potential caries protective action of the above fractions in human milk were in the following order: casein> whey protein> lactose> milk fat while in bovine milk: whey protein/milk fat > casein> lactose respectively.

REFERENCES

1. Grenby TH, Andrews AT, Mistry M, Williams RJH. Dental caries protective agents in milk and milk products: investigations on *vitro*. Journal of Dentistry, 29: 83–92, 2001.
2. Rugg-Gunn AJ, Roberts GJ, Wright WG. Effect of human milk on plaque on situ and enamel dissolution in vitro compared with bovine milk, lactose and sucrose. Caries Research, 19: 327–334, 1985.
3. Nelson, text book of Paediatrics. 14th edition, chapter 4, page 121.
4. Warner EA, Kanekanian AD, Andrews AT. Bioactivity of milk proteins: Anticariogenicity of whey protein. International Journal of Diary Technology, 54(4): 151–153, 2001.
5. Marthaler TM. Caries status in Europe and prediction of future trends. Caries Research, 24: 81–86, 1990.
6. Justin Merritt, Fengxia, Wenyuan. Milk helps build strong teeth and promotes oral health. CDA Journal, 34(5): 361–366, 2006.
7. Reynolds EC, Cain CJ, Webber FL, Black CL, Riley PF. Anticariogenicity of calcium phosphates complexes of tryptic casein phopshopeptides in the milk. Journal of Dental Research, 74: 1272–1279, 1995.
8. Shimazaki K. Lactoferrin: a marvelous protein in milk? Anim. Science Journal, 71: 329–347, 2000.