Bovine Milk: A1 and A2 Beta Casein Milk Proteins and their Impact on Human Health: A Review

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ABSTRACT

Milk is the ideal food for all age groups of human beings. Milk consists of 87 per cent water and the remaining 13 per cent is the milk sugar lactose, protein, fat, vitamins and minerals. Casein is the chief component of the milk proteins of which about 30 per cent is beta-casein. The major beta-casein variants are A1 and A2. Beta-casein consists of a chain of 229 amino acids. Cows that produce milk contain proline amino acid are called as A2 cows. A2 milk is found basically in indigenous cows and buffaloes of India. Around 5,000 years ago, a mutation occurred in this proline amino acid, converting it to histidine amino acid at 67th position. Cows that have this mutated beta-casein protein are called A1 cows. Different mutations bovine beta casein produce 13 genetic variants and out of these A1 and A2 are the most common. A1 β-casein is enzymatically broken down in the intestine to produce beta-casomorphin-7 (BCM-7) which is an opioid peptide similar to morphine. BCM-7 interacts with the human gastrointestinal tract, internal organs and brainstem. BCM-7 adversely affects the immune response and is also considered as a risk factor for chronic heart diseases (CHD) and juvenile insulin-dependent type I diabetes mellitus (DM-I). The original beta casein protein in bovine milk was A2. A2 is more comparable to the human beta casein than A1 in terms of digestive breakdown. Indigenous dairy breeds of cow (Red Sindh, Sahiwal, Tharparkar, Gir and Rath) and buffaloes produce A2 milk and India is endowed with rich A2 dairy animals.

Key words: A1 milk, A2 milk, Beta-Casomorphin-7, Human health.

Milk has been considered as a perfect food in all age groups including infants. Cow milk is closest to breast milk. It’s one of the inseparable parts of daily diet. Cow milk is a resource of fat, lactose, proteins, vitamins and minerals and also it contains several nutrients needed for growth and development. In health aspects, it contains immunoglobulin, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and several others bioactive peptides. Milk contains lactoferrin which is microbicidal. Cow milk is constituted of carbohydrate (Lactose) - 4.8 per cent, Fat - 4 per cent, Protein (Casein, Whey, Glycoprotein) - 3.5 per cent, Minerals (Ca, K, I) - 0.8 per cent and Vitamins (A, B2, B12) and supplies 66 kcal of energy per 100 grams whereas Human milk contains an average 1.1 per cent protein, 4.2 per cent fat, 7.0 per cent lactose and supplies 72 kcal of energy per 100 grams. Many cow products are used for their health promoting, disease-preventing and therapeutic purposes. Besides its nutritive use, milk and milk products are used in conjunction with medicines to enhance their pharmacokinetic and dynamic benefits. However an extensive body of evidence now exists that one particular type of milk containing specific beta casein protein might be harmful to the body.

Bovine Milk Protein: Beta casein

Bovine milk consist of about 87 per cent of water and the remaining 13 per cent milk solids constituting fat, lactose, proteins and minerals. Cow milk is generally contains about 3.5 per cent proteins, of which approximately 80 per cent are caseins (αS1-, αS2-, β-, κ-CN) and 20 per cent are whey proteins (α-La and β-LG). Casein protein becomes a major source for supply of all essential amino acids (except sulphur-containing amino acids - methionine and cysteine). Casein protein constitutes of 36 per cent, α-Casein, 30 per cent, β-Casein, 9 per cent κ-casein and 25 per cent peptides and amino acids. Among the caseins, beta casein is the second most abundant protein and has excellent nutritional balance of amino acids.

A1 and A2 milk

Milk that contains A1 β-Casein and A2 β-Casein are known as A1 milk and A2 milk respectively. Cows that produce milk contain proline (a specific amino acid) at 67th position are called as A2 cows i.e. the original breeds of cows. A2 milk is found basically in indigenous cows and buffaloes of India. Around 5,000 years ago, a mutation occurred in this proline amino acid, converting it to histidine (a different type of amino acid). Cows that have this mutated beta casein protein are called A1 cows. A1 protein variant is commonly found in...
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Bovine β-Casein has 209 amino acid residues, of which 16.7 per cent are proline evenly distributed along the polypeptide, which limits the formation of α-helix. Molecular weight of beta casein is 24KD. Different mutations have led to generation of 13 genetic variants of beta casein: A1, A2, A3, A4, B, C, D, E, F, H1, H2, I and G. Each variant differs from other variant in terms of amino acid substitution at fixed position.

The gene encoding beta-casein was changed such that the 67th amino acid in the 209 amino proteins was switched from proline to histidine. This may cause a change in the secondary conformation of the protein structure and affect the physical properties of casein micelle and vulnerability to enzymatic digestion. During this enzymatic process, beta-casein opioid peptide beta-casomorphin-7 (BCM-7) a decapetide is released exclusively from A1 and B variants. Depending upon the presence of proline and histidine at position 67, the other variants have also been categorized as A2 or A1 type.

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Proline has a strong bond to a small protein called BCM7 (Beta-Casomorphin-7)

A1 β casein is enzymatically broken down in the intestine to produce β-CM-7 which is an opioid peptide similar to morphine and named as β-casomorphins (β-CMs). It is a small protein that does not digest in human body. This can lead to indigestion and many types of research have shown that it may lead to various other problems or diseases like type-1 diabetes, coronary heart disease, atherosclerosis, sudden infant death syndrome, autism and schizophrenia. (Elliott et al. 1999; McLachlan 2001; Tailford 2003). In A2 milk, the proline amino acid found in 67th position of the β-casein prevents BCM 7 formation in our body. But, A1 milk containing β-casein variants that has histidine at the 67th position of the β-casein allows the cleavage at this position by different gastrointestinal enzymes to release BCM-7.

Proline has a strong bond to a small protein called BCM7 and therefore stops the BCM7 from getting into milk. So essentially, no BCM7 is found in the urine, blood or gastro-intestinal tracts of the original A2 cows. Histidine, the mutated protein, does not have a strong bond to hold on to BCM7. Hence, on consumption of the A1 milk, this protein BCM7 gets into the gastro-intestinal tract of animals and humans. A2 beta-casein milk, the milk proteins are broken down into peptides, which in turn are broken down into amino acids. This type of milk is easily digestible. However, in the case of the A1 beta-casein milk, the peptides cannot be broken down into amino acids and so, are

Fig 1: Difference between A1 and A2 beta-casein.
indigestible. These A1 beta-casein protein fragments that enter the bloodstream through leakages in the guts cause serious ailments, including coronary heart disease, Type I diabetes and autism.

The A1 and A2 variants of bovine beta-casein differ at amino acid 67th position with histidine in A1 and proline in A2 milk. This polymorphism leads to key conformational changes in the secondary structure of expressed beta-casein protein (Elliot et al., 1999; McLachlan, 2001). Due to presence of histidine at amino acid 67th position, digestion of A1 beta-casein milk releases a 7 amino acid bioactive peptide called beta-casomorphin 7 (BCM-7) in small intestine, while proline in A2 milk at 67th position prevents the split at this particular site and generates peptide BCM-9 (Roginski, 2003; Kostya et al., 2004). It is believed that generation of BCM-7 is the major causative factor associated with A1 milk related health disorders. However, A2 beta-casein not been linked to any of such health issues (Kaminski et al., 2007).

**Health Complications Linked with BCMs**

A1 type milk is suggested to be associated as a risk factor for human health hazards as it can potentially affect numerous opioid receptors in the nervous, endocrine and immune system. There are multiple strands to the evidence linking A1 beta-casein to different diseases. These include epidemiology, biochemistry, pharmacology, immunology, animal trails and human studies. Hence it is pertinent to study the effect of A1/A2 type milk on human.

Incidence of mortality rates chronic heart diseases (CHD) and juvenile insulin dependent Type I diabetes mellitus (DM-I) was reported in consumption of A1 beta-casein milk. (Elliott et al., 1999).

A significant relationship was observed between bovine milk protein consumption and the incidence of type 1 diabetes and CVD (McLachlan, 2001; Laugesen and Elliott, 2003) arteriosclerosis (Tailford et al., 2003). Besides, neurological disorders such as schizophrenia and autism (Woodford, 2006) and sudden infant death syndrome were also appeared to be known to potentiated by milk (Sun et al., 1999; Sun and Cade, 1999; Sun et al., 2003).

Jianqin (2016) reported that A1 beta-casein protein causes inflammation high in the small bowel (duodenum) which may decrease lactase expression lower in the small bowel (ileum) and manifest as Lactose Intolerance.

A human clinical trial conducted at Curtin University in Australia did prove that there were significant differences in digestive symptoms between milks containing A1 and A2 beta-casein. It has been approved that certain unwanted proteins or peptides that do not naturally occur in the human body may cause digestive disorders like irritable bowel syndrome or a weak gut.

Genetic variants in bovine beta-casein gene (A1 and B) release a bioactive peptide, beta-casomorphin-7(BCM-7) upon digestion, responsible for many human disorders like Type 1 diabetes, autism, schizophrenia and heart diseases but A2 milk does not cause such type of illnesses (Mishra et al., 2009; Sodhi et al., 2012). Infants may absorb BCM-7 due to an immature gastrointestinal tract. Adults, on the other hand, appear to reap the biological activity locally on the intestinal brush border. BCM-7 can potentially affect numerous opioid receptors in the nervous, endocrine and immune systems. Whether there is a definite health benefit to milk containing the A2 genetic variant is unknown and requires further investigation unlike harmful effects of A1 milk.

A number of studies have been published regarding the connection between diet and neurological disorders. The scientific reports confirm that in some children biopeptides of casein can leak through the gut wall into blood and from there into the brain causing significant behavioural problems. Investigators at the Florida postulated that BCM-7 reaches the brain cells and lead to symptoms associated with schizophrenia, autism and SIDS (Sun et al., 1999; Sun and Cade, 1999; Sun, 2003). The preliminary findings of study at University of Floridan showed that 95% of 81 autistic children had 100 times the normal levels of milk protein in

![Fig 2: Release of BCM-7 from A1 variant of beta-casein.](image-url)
their blood and urine. When these children were put on a milk free diet, at least 8 out of 10 no longer had symptoms of autism and schizophrenia. Same neural disorders have been noticed in rats dosed with varying levels of BCM-7. Bovine β-casein gene A1 release a bioactive peptide, β-casomorphin-7 (BCM-7) upon digestion, responsible for many human disorders like Type 1 diabetes, autism, schizophrenia and heart diseases but A2 milk does not cause such type of illnesses (Sodhi et al., 2001: Mishra et al., 2009)

**Beneficial effects of BCM-7**

Apart from negative health impacts of BCM-7, there are certain reports indicating the association of BCM-7 with positive health promoting effects such as Antagonist for 5-HT2-serotonin receptor and found to block serotonin induced platelet aggregation

- Inhibin of epithelial-mesenchymal transdifferentiation during hyperglycemia in NRK-52E cells by BCM-7 preventing, fibrosis in chronic renal failure.
- Contribution to mucin production in intestine which has protective function.
- Protective role in preventing high glucose induced oxidative stress in both in-vivo and in-vitro model.
- Decreased oxidative stress in pancreas of diabetic mice by inhibiting NF-kB-iNOS-NO signalling pathway.

In addition, several reports indicate nonsignificant changes in the morphology of blood cells, lipid profile (triglyceride, LDL and HDL), liver enzymes (alanine transaminase and aspartate aminotransferase), creatine and urea both with supplementation of A1 type milk/BCM-7 or due to increased activity of dipeptidyl peptidase IV, hence the in-vivo effects of A1/A2 like variants of β-casein remain controversial and warrant further validation. Considering public health related issues of β-casein A1/A2 variants and widespread use of taurine germplasm in our cross breeding programs for years and fact that these cattle could be the potential source for undesirable A1 allele of β-casein, warrant the need to analyze the status of β-casein/A1/A2 alleles in these animals to draw a sound breeding policy and minimize the risk of disseminating the A1 allele in Indian cattle. Our native Indian cattle breeds are natural source of A2 beta casein alleles, screening and selective distribution of the bull samples with desirable β-casein A2 genotypes will allow developing herds of cows producing milk with A2 variant only.

**CONCLUSION**

The A1/A2 hypothesis is both intriguing and potentially very important for public health. A few studies indicate that A1 beta casein may have adverse effects in certain individuals. A1 β-casein has histidine at 67th position while A2 β-casein has proline. The difference in amino acid at 67th position results in differences in susceptibility of peptide bond between amino acid 66 and 67. The peptide bond between isoleucine and histidine (A1 milk) is cleaved by elastase, while bond between isoleucine and proline (A2 milk) is not hydrolysed. The digested product contained a seven amino acid long peptide having sequence Tyr60 -Pro61 -Ph62 -Pro63 -Gly64 -Ile65 -Ile66 and is referred as betacasomorphin-7 or popularly called as BCM-7 which is not formed on digestion of β-casein A2. A1 β-casein was digested with combination of enzymes such as pepsin, elastase, leucine aminopeptidase (LAP) and pancreatin. Along with BCM-7, other degradation products such as BCM-9, BCM-13 and BCM-21 were also produced. BCM-7 is further breakdown to BCM-5 and BCM-3 by dipeptidyl peptidase IV (DPP IV) enzyme present on surface of enterocytes and in blood. The epidemiological and in-vitro data suggests the potential health hazards of consuming A1 type milk derived BCM-7, but the in-vivo studies of the same are very few to support the findings. Although a clear link between A1 β-casein and a disease state has not yet been confirmed.

The hypothesis of A1 milk association with few chronic diseases drawn from a survey done in western countries population not in India. Considering the contribution of buffalo, indigenous cows and crossbred population in India’s milk pool and based on the assumption that β-casein is 45 per cent of total protein and that around 25 per cent of the β-casein may be from A111 allele cows, the average consumption of A1 milk in India would be around 0.24g/day; 5-10 times lower than countries surveyed. So there is no cause to panic.

Indigenous dairy animals produce A2 milk. A2 Milk protects against diabetes, cardiovascular disease and neurological disorder and also increases the immunity of body disease. If there is demand from consumers for A2 milk as a matter of choice, the government of India and state governments should come up with policy guidelines and entrust the certification powers with the agency that is currently certifying organic milk, otherwise the consumers are likely to be rigid.

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