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Diet, Gut Microbiota and Its Association with Colon Cancer

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ABSTRACT

Probiotics and prebiotics are very necessary for the intestinal health of human. Probiotics may be naturally present in the individual's colon or it its growth may be enhanced by administrating adequate amount of prebiotics which favors their growth. Many factors like type of diet, quantity, its transit time and its pH determine the type, growth and efficiency of colonic bacteria. Probiotic ferment the dietary fibers and produces significant amount of many metabolites. Among these metabolites production of short chain fatty acids are very important as they plays many vital role in prevention of many diseases but recent research shows that they are anti-carcinogens and prevent colonic cells to mutate into cancerous cells. So dietary fibers and their corresponding bacteria save us from colorectal cancer. GSTs are important enzymes that prove helpful to increase immunity and act as chemoprevention in early stages of cancer.

Keywords: Acetone, Anti-Carcinogens, Butyrate, Colorectal Cancer, Fermentation, Glutathione S Transferase (Gsts), Probiotics, Propionate, Short Chain Fatty Acids.

I. INTRODUCTION

The microorganisms that are given in adequate amount to the human body having beneficial effects on human body are called Probiotics. These are live microorganisms [1]. Certain bacteria have been discovered in many years that have probiotic properties, which consist generally the bacteria that produce lactic acids are *lactobacillus*, *enterococcus*, *streptococcus*, *lactococcus*, *bifidobacterium*. Some Fungi are also used for this purpose like *Saccharomyces* and *Aspergillus*. Whereas the food material that is not digested with food but it should be major constituent of our diet is prebiotics. These prebiotics have many beneficially effects on human body such efficient growth activity of those bacteria that are beneficial for human health and are part of normal flora. Probiotics are necessary or intestinal health and its better activity so prebiotics present in our diet should not be absorbed in our stomach, so it must reach the lower part of our body such as colon to promote the growth of probiotics [2]. Probiotics have many beneficial effects on whole digestive tract and make it healthier. These also help in improving immunity. We can also treat the patients having lactose intolerance by these prebiotics. The biotransformation of isoflavone phytoestrogen to improve post-menopausal symptoms, bioconversion of bioactive peptides for anti-hypertension, and reducing serum cholesterol level can be done by wisely using them [3]. There are many bacteria that are considered as probiotic bacteria having different beneficial effects on human health [4]. These may be to cure the adverse effects of lactose intolerance and for the relief of cancer, vaginitis and high cholesterol level. Today, these are used to reduce the side effects of anticancer drugs in case of colorectal cancer. The researchers are trying to discover use of probiotics in the case of breast and bladder cancer. But now CRC is on the second number cancer having high mortality rate in Europe, where it's early diagnose in too poor [5].

So it is so easy to prevent this disease before its happening. Epidemiological study shows that there are many factors that plays a vital role in most large bowel cancers mainly diet of a person. This disease is higly curable disease just changing our diet and life style. Alteration in normal micro flora of intestines causes the onset of colon cancer. So this change promotes the development of tumor. Fermented milk products are very useful to prevent such disorder as these products consist a large number of bacteria producing lactic acid which help to maintain a well-balanced micro flora. So, if a person has a good and healthy intestinal microflora it will prevent from diarrhea, lactose intolerance and many

other immunological diseases [6]. The individuals taking a large proportion of fermented milk products like curd having a low chance of colon cancer [7]. This defensive mechanism of fermented milk is observed in lab experiments in which many LAB inactivates the carcinogens. So, this can be use as its preventing agent [8].

II. METHODS AND MATERIAL

1. Diet and the Composition of the GUT Microbiota

Colonization of microorganism changes according to the different properties of gut which vary continuously change of pH, nutrient and oxygen availability, body secretions like bile and all digestive enzymes. The different parts of the gastrointestinal tract fluctuate widely in terms of transit time, pH, nutrient availability, exposure to oxygen, mucosal surfaces, host secretions (such as bile and digestive enzymes). Immune system is also very important in the colonization of microflora [9]. In a healthy individual large intestine is the part where a immense number of most efficient and beneficial bacteria are present. These bacteria are metabolically active and their number is more than 1011 cells per g. In intestinal microflora the number of anaerobic bacteria is very high. These anaerobic bacteria mainly belongs to phyla which mainly includes the *Bacteroidetes* and *Firmicutes* (Eckburg *et al.,* 2005)[10]. The composition of gut microbiota changes according to the diet that is taken by an individual. It is noticed that diatery compostion has an influence on changing of microbiota of fecal material [11]. The number of *Firmicutes* and *Actinobactera* becomes high if we intake carbohydrate rich diet [12]. if we take starch rich diet then the *Firmicutes* specially, *Ruminococcus bromii*) increase. Wheat bran-enriched diet favours the growth of *Lachnospiraceae* increase and causes decrease in bacteria that produce the butyrate. It is observed that weight loss diets having low carbohydrate level cause the change in *fecalmicrobiota* [13].

It is clearly noticed that more intake of plant based diets means fibre and low levels of protein and fat (approximately 32% and 10% calories) favours growth of *Firmicutes* while animal base diet rich in proteins and fats favours the *Bacteroidetes* and decreased in *Firmicutes* [14]. This shows that *prevotella spp*. has excellent ability of degradation of fibre than *Bacteroides*. *Faecalmicrobiota* has a bimodal distribution when we study it in a population by metagenomic analysis. Its mean some persons have very low diversity having low gene count(LGC) while other healthy individual have a high gene count(HGC). Production of butyrate produced by *Firmicutes* becomes low in Communities with LGC. So it increases the chances of obesity and metabolic syndrome [15]. Some this type of obesity is cured by giving weight loss diet to the persons having LGC. By this the microbial diversity increases and will reach to community with HGC [16]. So, diet plays a vital role in formation of normal flora of digestive tract Thus, our diet rich in fibre prevent the cell for susceptibility to diseases like CRC. By recent studies it is observed that all individuals with low fibre intake (like cereals and whole grain are at high risk of CRC and colorectal adenomas while comparing with healthy individuals [17].

High consumption of fats, red meat and alcohol favours CRC. Non digestable carbohydrates favours and improve the fermentation ability of intestinal microflora and it is observed in rural native Africians having low ratio of CRC when compared with African America with poor fermentation of gut microbial flora. Onset of colorectal cancer is caused by some toxic and harmful metabolites by dietry components and environment [18].

2. Metabolism of Probiotics in GUT

When an individual intake undigested dietary components it is fermented in large intestine. Various types of metabolites produce by the fermentation of anaerobic bacteria present in gut. So these metabolites will be chemically different due to the different availability of food which acts as substrate. It shows an extraordinary biochemical ability of this microbiota [19]. The end product of fermentation in healthy persons is different than others. There are three main and important short chain fatty acids (SCFAs) includes acetate, propionate and butyrate, besides these gases are also produced as in fig. 1. This process of fermentation is take place in colon. Many un-digestible carbohydrates like polysaccrides and some oligosaccrides serve as primary substrate for fermentation process. These carbohydrates are the basic structural carbohydrate of plant cell wall. fructo-oligosaccharides is a soluble oligosaccrides which promotes this fermentation process [9]. The physiology of colon changes widely according to the diet habit and its time.

Dietry habits in which diets constituents and its time is very important which directly influence availability of of non-digestible fibre. So according to it free carbohydrate will be available in the colon. Bacteria in colon respire anaerobically as various organic compound and nitrates and sulphate act as electron acceptor. So bacterial metabolism in colon is not purely fermentative [20]. *Proteobacteria* is more successful to generate energy for them due to having ability of using free oxygen as electron acceptor. They can generate energy using substrate better to obligate anaerobes. *Bacteroides spp.* has cytochromes for this purpose. It is observed that F. *prausnitzii* depends upon extracellular electron transfer by flavins and thiols. Microbes in colon are anaerobic and required very low concentration of oxygen so this condition will favors the anaerobic flora there. According to that condition the ecology of gut decide the bacteria suitable for that area [21]. Many bacteria have cross-feeding interaction as end product of metabolism of one specie used by others. So they favor the growth of others. Like hydrogen, formates, sulphate reducing bacteria have an important role in anaerobic repiration on colon. The abundance of methanobacteria is different in gut according to type and time of gut. So these bacteria will contribute in determining of acetogenesis, sulphatereduction and methanogenesis [22].

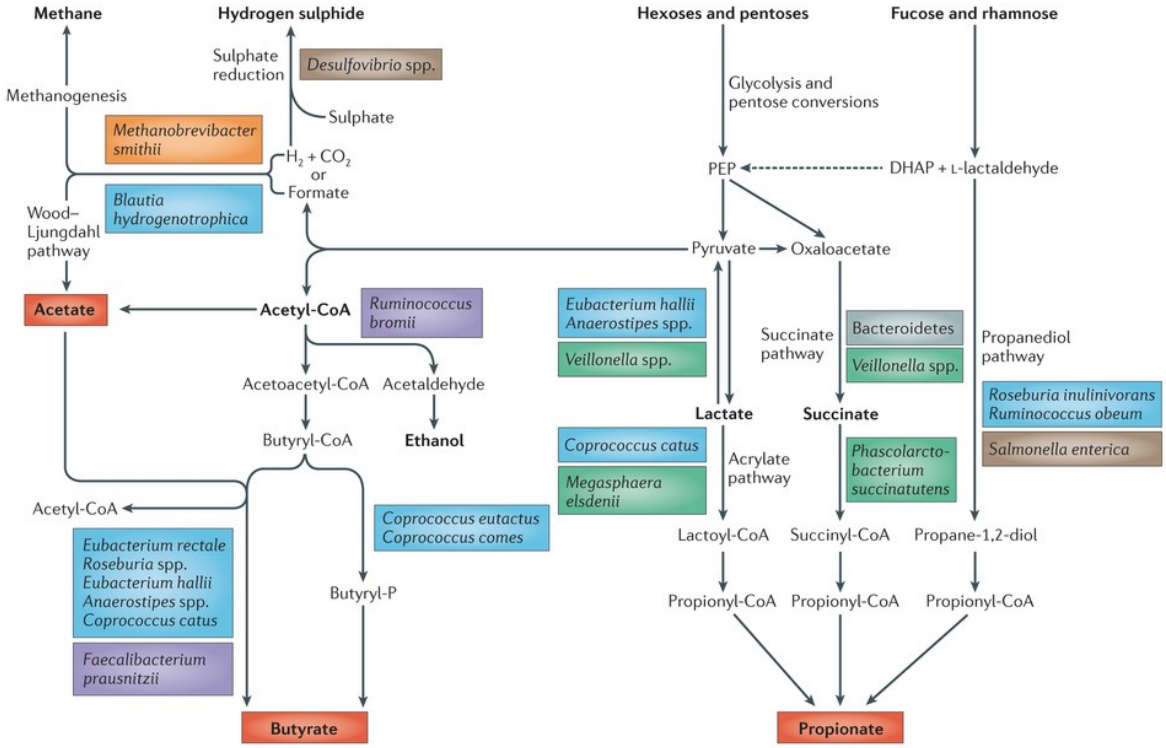

Figure 1. Fermentation of fibers and other cross-feeding pathways that decides the pathways for synthesis of microbial metabolites

3. SCFA Productions

Dietary fibers help in laxation, mineral absorption, potential anticancer properties, lipid metabolism and anti- inflammatory effects [23]. The end product of microbial fermentation in colon is SCFAs as in fig.3. This whole process is represented by the following equation [24].

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$$2C6H12O6 + 38\ H2O \rightarrow 60\ \text{acetate} + 22\ \text{propionate} + 18\ \text{butyrate} + 96\ CO2 + 256\ H+.$$

In this equation major SCFs acetate, butyrate and propionate is formed by the fermentation of carbohydrates. This process is very important for carbohydrate metabolism as we intake more carbohydrates there will be more production of these bacterial mass which increase the health and activity of intestines. The large bacterial mass also act as laxative and decrease the transit time of colon. The reduction in

transit time also reduces the breakdown of protein. Thus it will lead to accumulation of ammonia, amines, hydrogen sulfide and phenols in the colon. The total concentration of these SCFs exceeded 100mmol/L in colon [25]. The molar proportion of SCFs depends upon normal flora of gut and nature of diet. Usually, proportion of acetate is more than others as it makes about 60%-75% of the total SFCA. This acetate is produced by many bacterial groups that are normal resident of colon. By process of reductive acetogenesis about 1/3 part of acetate is formed [26].

Propionate and butyrate have also many healthy effects on colon. These are produced by some specified bacterial species. These specified bacterial species provide wide molecular functions that is very important to know the function and composition of normal flora of colon [27]. It is observed that 2 new propionate producing bacteria in colon are emerging which have many pathways for the biosynthesis of propionate. Bacteroides species facilitate the conversion of succinate to propionate. The clostridial cluster IX group adopts the acrylate route from lactate. There is also a third pathway followed by butyrate producing bacteria use fructose as substrate [28]. *Faecalibacteria* and *Eubacterium* are the abundantly found bacteria in gut consisting 7%-24% of total flora of gut. It is observed that obese persons that take low dietry fibres have reduced level of butyrate producing bacteria and butyrate which relates to *Eubacterium* rectal and *Roseburiaspp* [29].

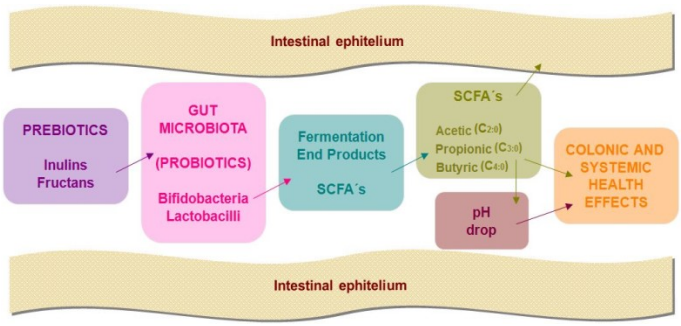

Figure 3. Production and role of SCFAs in colon

4. Factors That Affect SCFA in the Colon

Metabolism of readily digestible carbohydrate takes place in proximal part of large intestines with depletion of carbon source. With the progressively reduction in available substrate for bacteria the food residues moves towards distal part of intestines. The lengths of time for which this material stay in digestive tract determine the type and amount of SCFAs in colon [30]. The physiology and metabolism in large intestine influence the protein breakdown. Amino acid fermentation increased production of SCFAs in colon like a pool observed by in vitro and in vivo studies [31]. In the very proximal part of large intestine due to the large availability of carbohydrates the production oh SCFAs is at peak. Subsequent work demonstrated many important aspects like the metabolites produced by these bacteria are absorbed in colon. We can also conclude the formation of SCFAs and their balance in the bowel contents. The bacterial fermentation products in the colon are widely distributed in the different parts of colon. This distribution is both qualitative and quantitative; it is commonly in relation to butyrate and other products of dissimilatory amino acid metabolism [32]. There are many factors other than transit time of gut which influence the metabolism of bacteria. These factors are of the host's itself which have very deep impact on the production of SCFAs in colon as in fig. 3. These factors may be the nature of diet and many others like ageing, pancreatic activity, endocrine system, secretions of gut. Besides these, mucus production in gut, stress, disease, drugs and antibiotics can cause change in the fermentation as it disturb the normal flora of the gut so, it will also disturb the production of SCFAs formation. Bacterial variety and their number in the colon chemical composition of diet, physical form and available substrate quantity these are also influenced the fermentation reaction. These all factors also influenced the catabolite regulatory mechanisms and availability of inorganic electron donors, like nitrate and sulfate [33]. In the normal flora of gut there are different associations between them. This may be competitive in which they assist and beneficial to one another or may be competitive in which they compete for their survival [34].

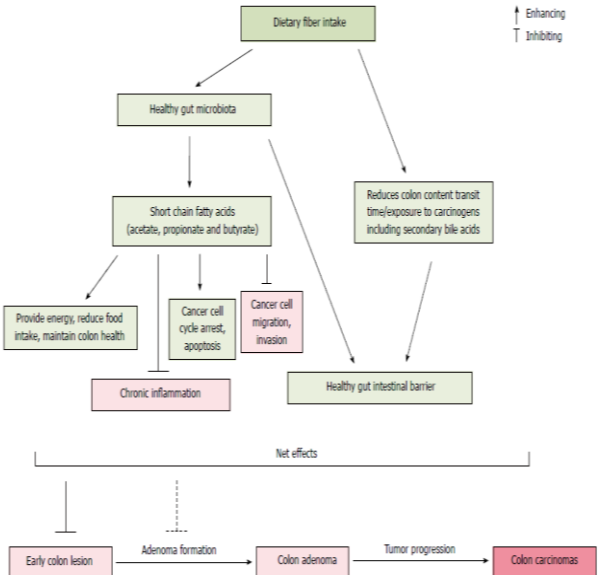

Figure 3. Primary pathway of consumption of dietary fiber, gut normal flora and its link with colorectal cancer

III. RESULTS AND DISCUSSION

1. Physiological Effects of SCFA

Three important SCFAs are found mainly in the colon of human intestine. These are Acetate (C2), propionate (C3) and butyrate (C4). When the synthesis of these SCFAs takes place they are released in the lumen. These SCFAs are main source of energy for the cells of colon when they get absorbed by the cells as in fig. 4. It fulfills more than 10% of basic energy requirements of the cell. Besides colonocytes, they also absorbed by liver and muscles [35]. Effect of acetate on rabbit smooth muscles of colon was studied which showed that it has positive effect on the proliferation of cell while it has negative effect on spontaneous longitudinal muscles [36]. Ileal motility is enhanced as acetate enhances. Because acetate enhances the blood flow in the colonic cells. It is also important to provoke the immunity of the host cooperating with receptors of G protein coupled in immune cells and adipose tissues of liver and kidney. It is also plays a vital role in adipogenesis [3]. Acetate as also reduces the chances of cancer by enhancing antibody production in peripheral blood of many tissues. Acetate reduces the lipopolysaccride level in blood. It also stimulate the tumor necrosis factor (TNF), nuclear factor(NF) and interleukin (IL) [37]. Concentration of propionate has also effect on muscle contractions by enteric nerves. These contractions were of longitudinal muscles of distal colon of rat similar as action of acetate. Propionate plays an important role in the satiation of organism as it help to release the leptin hormone. Thus it reduces the intake of food by activating leptin of GPCR43,41 [38]. Propionate is protective against carcinogenesis. It stimulates the apoptosis in colon. It stops the growth of cancer cells of colon and the differentiation of normal cells into the cancerous by process of hyperacetylation of histone protein [39].

Propionate also inhibit the massive production of cytokines which are important in proinflammatory symptoms by activating TNF- α, NF-κB in various tissue. Some epithelial cells metabolized these SCFAs to obtain energy. Butyrate is the most important in maintaining the colonic health. It also moderate the cell growth and its differentiation [40]. Butyrate is also very important in uptake and consumption of oxygen. On isolating the colonic cells. It was observed that oxidation of butyrate invites the cells to the uptake of oxygen. Due to that reason it was seen that the concentration of butyrate was very high up to 70% in the isolated colonocytes. Butyrate level is found very high on analyzing the colonic content in arterial and portal blood. Besides this, butyrate also shows a powerful anti-inflammatory characteristic as compared to acetate and propionate. This is due to activation of various interleukins and nuclear factors. These all expressed in the epithelial cells of colon and immune cells [41].

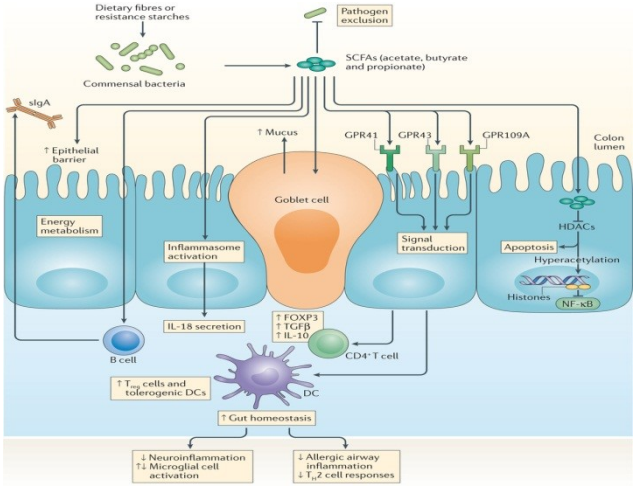

Fig. 4. Synthesis of SCFAs, its effect on physiology and immune system of host

2. Mechanism of Primary Cancer Prevention by Butyrate

As described earlier, butyrate has a vital role in the chemoprevention. It activating the glutathione S

transferase (GSTs) by SCFA. It is most effective mechanism of chemoprevention by detoxify various carcinogens. GSTs are enzymes which can biotransformed [42]. Increased level of this enzyme act against genotoxic compounds in cell lines of tumor derived. These genotoxic compounds are food derived it may be 4-hydroxynonenal (HNE). It is also effective in non-transformation cells because it decrease the chances of cancer initiation. Its mean it is an effective chemoprevention in early stages of cancer [43]. Toxification in produced by smoking can be neutralized by GSTs. These enzymes act as anti-carcinogens [42]. Effect of butyrate on gut by the fermentation of fibres present in diet is most significant and there are many papers that compile its effects on the human by experimental studies. This process is usually carried by use of inulin by observing its effects on cells of colon. But now further research is going to find new ways by focusing on modulation of genes and its expression related to these aspects [44].

IV. CONCLUSION

Diet is the major factor that effects the presence of gut normal flora and its number. In broad term, substrate availability favors the composition of species of bacteria. Fibers in food favor the beneficial bacteria that are necessary for human intestinal health. The stay time of food in the gut decides the kind and quantity of SCFAs in the healthy adults. So, intake of more dietary fibers in diet helps to prevent the colon cancer. These fibers are non-degradable in proximal part of intestine and finally reach to its destination where fermentation takes place by probiotics.

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