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Review

Gut Microflora and Atherosclerotic Cardiovascular Disease: A Review

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Abstract

Atherosclerotic Cardiovascular Disease (ACVD) is one of the leading causes of death throughout the world. ACVD is inflammatory and occurs due to the deposition of lipids in the arterial lumen. The increasing trend of ACVD can be attributed to the sedentary lifestyle and it is imperative to investigate the causes of ACVD and their prevention. The human gut hosts an enormous diversity of microbes. This enormous microbial community inhabiting the human gut are responsible for several conditions associated with ACVD and plays a crucial role in its progression. Different methods for studying gut microflora have been developed, which has led to the discovery of dysbiosis. Dysbiosis is the change in homeostasis of the microfloral community. Several external factors are responsible for the dysbiosis including diet, mode of delivery, age, sex, body mass index, host genetics, and antibiotic usage. Dysbiosis results in the release of altered amounts of some bioactive metabolites such as short-chain fatty acids (SCFA), bile acids, and trimethylamine-N-oxide (TMAO) which can be a contributing factor in the progression of atherosclerosis. This review will enlist the role of dysbiosis of gut microbiota in atherogenicity and their relevant applications in its prevention. With an adequate understanding of the process involved in gut microbiota dysbiosis, scientists worldwide can develop potential therapeutics for ACVD.

Keywords-: Atherosclerotic Cardiovascular Disease (ACVD), Dysbiosis, Gut microbiota, TMAO, SCFA.

Introduction

Cardiovascular diseases, including atherosclerotic cardiovascular disease (ACVD), hypertension, heart failure, and heart stroke, are the leading causes of death in different nations. According to World Health Organization (WHO) reports, cardiovascular diseases were responsible for around 32% of the world's total death in 2019 [1]. Atherosclerosis is known to have serious ill effects on a person's health and reduces life expectancy. An increase in modern-day facilities has promoted a sedentary lifestyle, which has proven to be a significant risk factor for ACVD [2]. There is an ever-growing demand for developing the latest therapeutic strategies for ACVD, heart failure, and hypertension. It is expected that the current therapeutic strategies will not be sufficient for the growing cases of ACVD in the next decade [1, 3].

Atherosclerosis is an inflammatory disorder caused due to the deposition of oxidized low-density lipoproteins (ox-LDL) and dead cells in the arterial lumen [4, 5]. The other contributing factors of ACVD include the formation and deposition of foam cells in the tunica intima of the artery. This foam cell formation requires the deployment of large numbers of monocytes and their further differentiation into tissue macrophages. These foam cells, along with ox-LDL, form plaques [6]. These plaques reduce the amount of blood reaching the coronary arteries and cause coronary artery disease (CAD) [7]. CAD generally leads to angina pectoris and chronic heart attack. ACVD can be controlled by obtaining information about the gut microflora and the metabolites produced by them.

The immune pathways are activated by the inception of various substances released by gut microflora. Macrophages are activated by the signals originating from these gut inhabitants. Lipopolysaccharide (LPS) is found in Gram-negative bacteria, which can activate these body defense pathways. LPS binds to Toll-like receptors (TLRs) and leads to the activation cascade of inflammatory processes [8,9]. Additionally, the gut microbial species produces various metabolites. These metabolites react with immune cells like the macrophages and facilitate the production of cytokines. Prolonged inflammation in such cases can trigger atherosclerotic disorders. Gut microbiota by mediating inflammation can be a contributing factor in the progression of ACVD.

The inappropriate gut microbiota increases atherogenicity by the release of bioactive metabolites [8,10]. Some gut microbes secrete enzymes that degrade the undigested carbohydrates and convert them into short-chain fatty acids (SCFA), which are critical inflammatory markers in humans [11]. Trimethylamine-N-oxide (TMAO) is a bioactive molecule synthesized coactively by humans and the gut microbial species. TMAO has been widely stated as a metabolite linking gut microbiota and ACVD, causing atherosclerosis [12]. These metabolites are produced as a result of prolonged dysbiosis of the gut. Investigation of the gut microflora community can be of great significance in the treatment of ACVD.

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Microfloral diversity in the human gut

Gut microflora (microbiota) is a group of microorganisms inhabiting the gastrointestinal regions of an organism. It is a group of cohabiting mutualistic, commensal, or pathogenic microbes within a host organism [13]. The human gut hosts trillions of these microbes, and they together form the gut microbiota. The majority of the human gut microbiome includes the bacterial species from the five phyla: *Firmicutes, Proteobacteria, Verrucomicrobia, Actinobacteria,* and *Bacteroidetes* [14-16]. Among these five phyla, *Firmicutes* and *Bacteriodetes* constitute about 90% of the microflora present in the human gut [14]. The relative abundance of these microflorae is given in Figure 1. These phyla include several species which have their role in moderation of gut environment. Most microbial classes are similar in the human gut; however, they still account for some differences [17,18]. Table 1 describes these characteristics of the majority of the microfloral phyla. The alteration in the diversity of microflora in the gut can lead to various diseases including atherosclerotic disorders, heart failure, heart attack, and hypertension [19-22]. The diversity of these microbes can be used as an important marker for checking the progression of several diseases. It is observed that the ratio of *Firmicutes* to *Bacteriodetes* is much larger in the patients suffering from ACVD [23]. Specific gut microfloral species protects from pathogenic invasion and positively affects the host body [24,25].

Table 1: Relative abundance and characteristics of major phyla of gut microflora.

S. No	Phylum	Relative Abundance	Characteristics of the Phylum	Major Genera	References
1.	*Bacteriodetes*	30%	Gram negative bacteria, Mainly present in gut, *Bacteriodetes* decreases in patients of CAD	*Bacteriodes, Prevotella, Parabacteriodes, Tannerella, Sphingobacterium*	[56,71]
2.	*Proteobacteria*	2%	Gram negative bacteria, Increased in atherosclerotic plaques,	*Helicobacter, Chrysemonas, Escherichia, Bilophila, Shigella*	[56,71]
3.	*Firmicutes*	65%	Gram positive bacteria, Abundant in gut, oral cavity and atherosclerotic plaques, Ratio of *Firmicutes* to *Bacteriodetes* is higher in patients of ACVD	*Clostridium, Lactobacillus, Roseburia, Eubacterium, Enterococcus*	[20,56,71]
4.	*Verrucomicrobia*	2%	Gram negative bacteria, Mucus degrading bacteria, Decreases in people of higher BMI	*Akkermansia,*	[50,71,72]
5.	*Actinobacteria*	1%	Gram positive bacteria, Present in gut, plaques and oral cavity, Prevalent in Vaginal delivery	*Corynebacterium, Bifidobacterium, Atopobium, Collinsella, Streptomyces*	[56,42,71, 73,74]

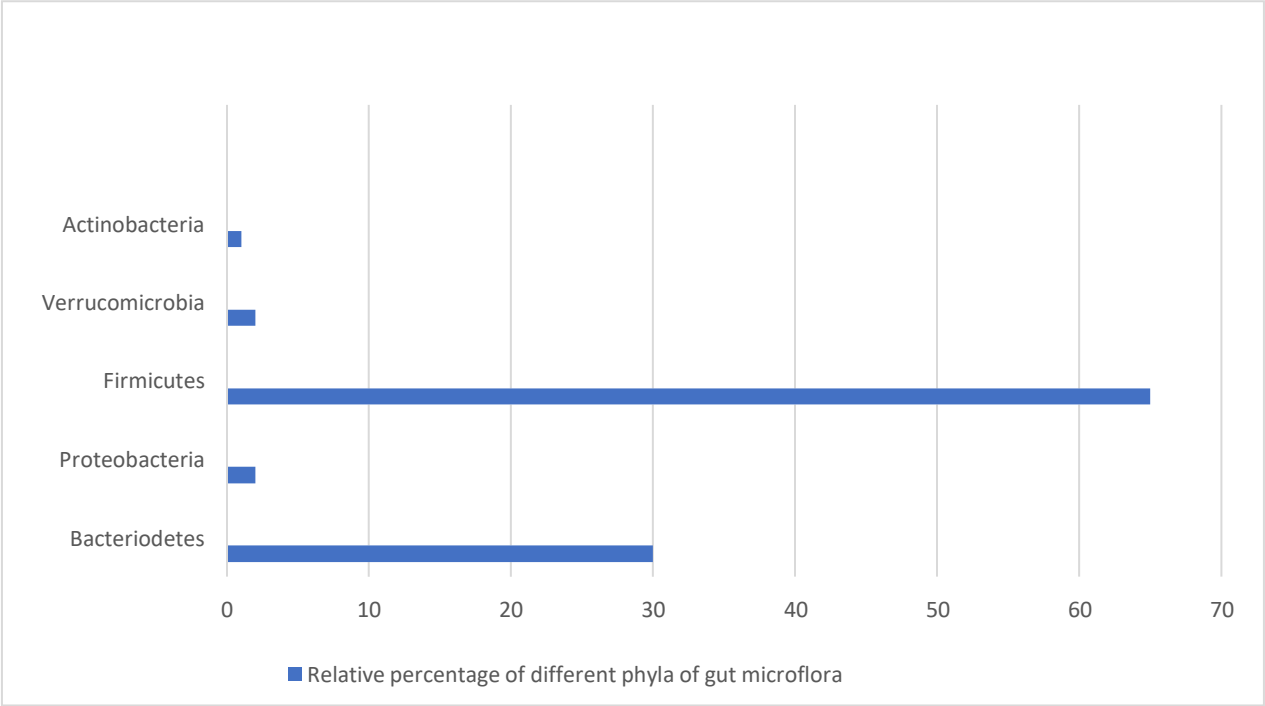

Figure 1: Relative Percentage of Different phyla of gut microflora. *Firmicutes* account upto 65% of the total microflora present in human gut. *Bacteriodetes* is the second largest phylum involving upto 30%, *Verrucomicrobia*, *Proteobacteria* and *Actinobacteria* accounts upto less than 5% of the total microflora [10, 11].

Methods of studying gut microbiota

Several methods are employed for studying gut microfloral species over time. These methods have undergone a paradigm shift in the past years. Figure 2 indicates the changes in the procedures for studying microflora. Culture-based methods are one of the conventional methods used for this purpose. The methods are labour intensive; hence, they are less frequently used. DNA-based methods are prevalent these days, which include sequencing and non-sequencing techniques [26]. Non-sequencing methods commonly used in the laboratory are fluorescence *in-situ* hybridization (FISH), flow cytometry, pulse-field gel electrophoresis, denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, and single-strand conformation polymorphism. Sequencing methods for studying microflora species involve 16S rRNA sequencing of hypervariable regions [27], metagenome sequencing, and meta-transcriptome sequencing.

Different sampling methods have evolved over the time for estimating the gut microflora. It includes direct evaluation from the patient's feces, which is followed by DNA extraction, amplification, and gene sequencing. The sequenced data is used to identify the microbial species [28]. Other sampling methods used variedly are endoscopic biopsy [29], luminal brushing [30,31], laser capture microdissection [30], and surgery [32]. Some ingestible sampling devices can also be used, especially targeted towards drug delivery and collection of gut fluid samples [33]. The sampling methods used should not cause any disturbance to the gut inhabitants.

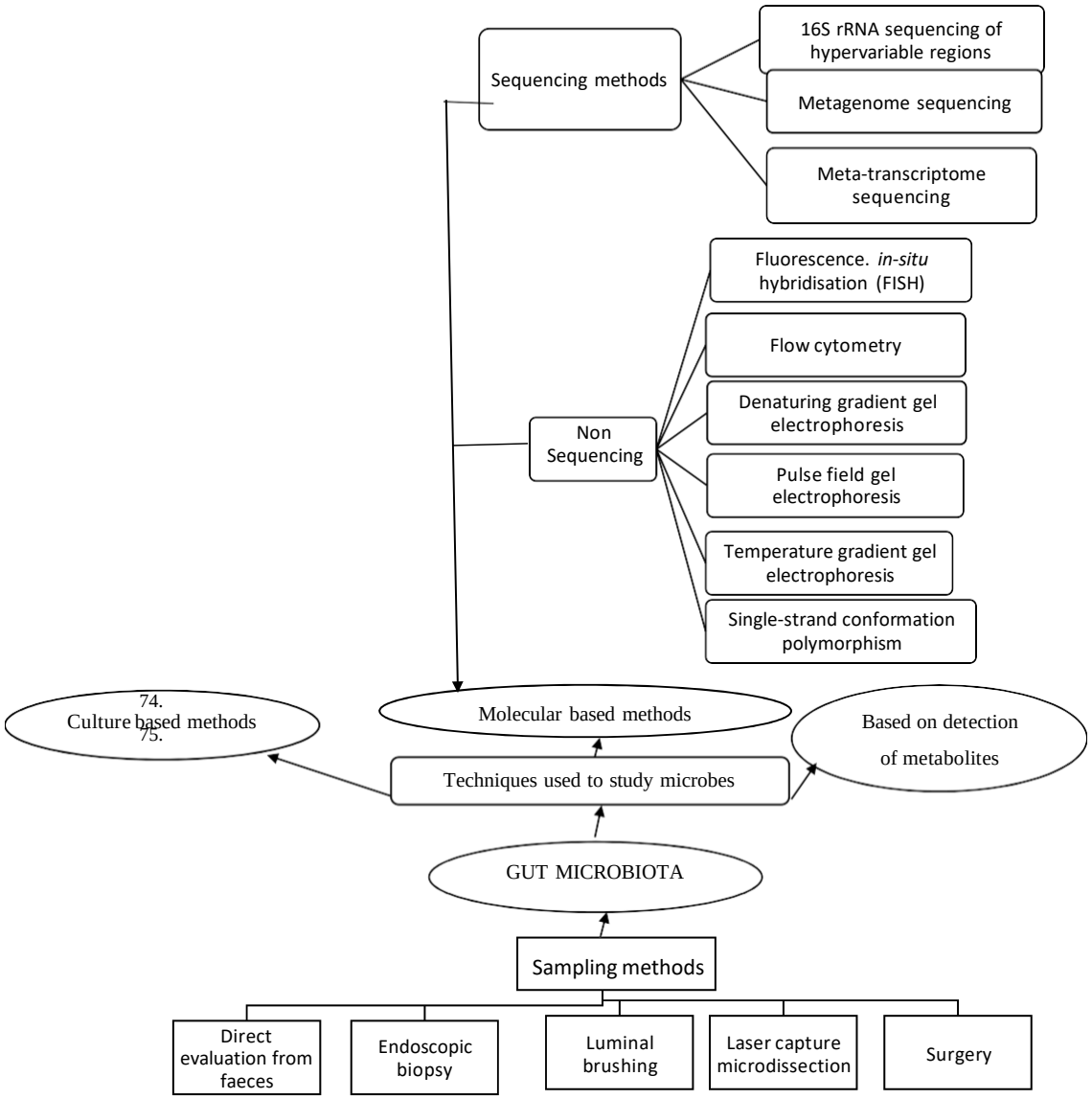

Figure 2: Techniques and Sampling methods used for studying microflora.

Manipulations of gut microflora by external factors

Human gut microflora developed at a very early age of the host. On an average, an adult human being has approximately 100 trillion bacteria present in its gut [34]. Though it somewhat remains constant, there are several factors that can contribute to dysbiosis. This dysbiosis results in the release of abnormal amounts of gut microbial metabolites, and thus, it contributes to ACVD. The dietary habits of a person are the primary factor, which describes his gut microflora. Other factors also exert their effect on the manipulation of alimentary canal microbiota. These factors include physical exercise, antibiotic usage, host's age, sex of the host, host genetics, and host diet (Table 2).

Diet

Diet is one of the integral factors for shaping one's microfloral species. It can act in both ways, either beneficial or harmful [35]. For example, foods rich in lipids and fats acquire Trimethylamine (TMA) in the form of phosphatidylcholine (PC) (lecithin), choline, and L-carnitine. Humans cannot degrade this TMA present in the diet due to the absence of TMA lyases. This TMA later gets oxidized to Trimethylamine -N- oxide (TMAO). TMAO is seen as a significant bioactive metabolite whose levels are increased in patients with ACVD. For instance, foods rich in PC such as pasta, meat, rice, and fishes contribute to increased levels of TMAO, a precursor for ACVD [36,37]. Vegetarian habits lead to the majority of *Firmicutes* and *Bacteroidetes* in the gut. The levels of *Prevotella* increase if a person's diet is plant-based [38]. Several human studies have been done stating that a diet

rich in starch can promote the growth of *Bifidobacterium*, which helps in the degradation of starch [39]. A protein-rich diet accounts for the higher amounts of *Bacteroides*, *Bilophila*, and *Alistipes* in the human gut [28,40,41]. Such diets are also responsible for the suppression of the *Firmicutes* [42]. It was further reported that *Prevotella* and *Alistipes* were exhausted in ACVD [22]. This indicates that a diet rich in proteins promotes healthy microflora and retards the progression of ACVD.

Delivery mode and age of the host organism

Delivery mode and age of the host organism determines the gut microflora species. These changes in the gut microflora are responsible for the secretion of abnormal quantities of the bioactive metabolites, which in turn play a positive role in ACVD progression. The method of delivery of a baby promotes a specific type of microflora. Vaginal births have an increased population of some genera, including *Lactobacillus*, *Prevotella*, and *Sneathia* [43]. Researchers have reported that *Lactobacillus* was elevated in patients with ACVD [15]. Those born with cesarean section have increased amounts of *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* species [43]. With advancing age, the number of these gut microbes increase. Children of age group 2-5 years have a majority of *Firmicutes and Bacteriodetes* [44], while the children of age group 11-18 years have the prevalence of *Clostridium* and *Bifidobacterium* genera [45]. Individuals of more than 60 years of age have significant amounts of *Bacteriodetes* [46].

Table 2: Microfloral variations with respect to different factors.

Factors Affecting Gut Microflora		***Firmicutes***	***Actinobacteria***	***Proteobacteria***	***Bacteriodetes***	***Verrucomicrobia***	**References**
Age	2-5 years	↑			↑		[43]
	11-18 years	↑	↑				[44]
	More than 60 years				↑		[45]
	Protein rich diets	↓		↑	↑		[27,39-41]
Diet	Plant based diet	↑	↑		↑		[37,38]
	Male	↓					[47]
Sex	Female	↑					[47]
BMI	Overweight	↓	↑		↓		[47,75]
	Underweight	↓		↑			[47,75]
Host genetics	Heritability	↑	↑				[48]
	Non-Heritability				↓		[48]
Physical exercise	High performance sports	↑		↑	↓	↓	[49,75]
	Regular exercise	↑	↑			↑	[50,51,75]
Antibiotics use	Vancomycin	↓		↑	↓		[43,54]

Host sex and body mass index (BMI)

The sex of the host organism also influences the relative abundance of the microflora species. The sex of the host is attributed to different lipid metabolic mechanisms and is observed in male and female mice [47]. Gender differences do not account much for the difference in gut microbial phylum level. However, minute differences have been observed at the level of genus [48]. Gender differences in gut microfloral species are synergistically related to BMI. When BMI increases, the effect of sex variation in gut microflora becomes more profound [48]. The *Firmicutes* are abundant in females irrespective of their BMI, but in males, individuals over the BMI 33 have a relatively lower abundance of *Firmicutes* [48].

Host genome

Host genetic make-up is critically involved in immunity and susceptibility against a disease. Human gut microflora has been associated with the host's innate immunity by its action on Pattern recognition receptors (PRR). It plays an indirect role in the progression of ACVD. The host genome is responsible for dysbiosis and thus, promotes the advancement of ACVD. Particular genes and their interplay with gut microflora are accountable for posing a threat in terms of ACVD. For example, it has been reported that possession of the rs651821 variant of the gene APOA5 endowed an individual with members of the genera *Methanobrevibacter*, *Lactobacillus,* and *Sutterella* in their microbiota, which shows a positive correlation with a greater risk of ACVD [49]. There is a higher probability of inheriting particular genera of microbes. The *Firmicutes*, *Actinobacteria*, *Tenericutes*, and *Euryarchaeota* are more heritable, whereas the abundant *Bacteroidetes* phylum shows very little or no heritability [49].

Physical exercise

Physical exercises have been proven beneficial for increasing the gut microbial diversity. The protein intake correlates with the diversity of gut microflora. There is an enhancement in relative numbers of *Clostridium, Haemophilus, Eisenbergiella*, *Faecalibacterium,* and *Sutterella* in bodybuilders in comparsion to non-body-builders [50]. Certain bacterial groups show enhanced growth in response to exercise, such as *Lactobacillus, Bifidobacterium,* and *Akkermansia* [51]. The bacterial groups that exhibit a decreased pace of growth in response to physical activity are *Proteobacteria*, *Turicibacter,* and *Rikenellaceae* [51]. Physical exercises do have acute and chronic effects on gastrointestinal microbes. Acute effects of physical exercise on gut microbiota can be seen by an increase in amounts of *Lentisphaerae* and *Acidobacteria* at the genus level and *Coriobacteriaceae* and *Succinivibrionaceae* at the species level. Some of these are involved in bile acid metabolism; hence, they increase binding terms [52]. Physical exercises can modify the gut microbiota and reduce the changes that contribute to ACVD [53]. Thus, it can play an essential role in preventing such diseases.

Antibiotics use

Antibiotics can kill microbes, but they cannot distinguish between the beneficial or the harmful bacteria. When antibiotics are administered, they kill bacteria regardless of their functionality. Due to this reason, they are often regarded as a double-edged sword [54]. When antibiotics are given, they kill the gut microbial population and thus, can cause dysbiosis. For instance, the antibiotic Vancomycin, when taken, reduces the amount of *Bacteroidetes*, *Fuminococcus,* and *Faecalibacterium* but increases the *Proteobacteria* species [44,55]. The effects of antibiotics are more severe when administered for a longer duration of time. This indicates that the ill effects of antibiotic use are dependent on the dose length [44]. This effect also depends on the type of antibiotic administered. It takes a longer time for the gut dysbiosis caused by the use of antibiotics to replenish itself, and it can be a causative factor in the progression of ACVD.

Bioactive microbial metabolites and their role in ACVD

Trimethylamine-N-oxide (TMAO)

TMAO is the most notable bioactive metabolite produced by gut microbes that possess the ability to modulate the ACVD progression. TMAO is produced when a diet rich in trimethylamine (TMA) is taken. TMA is present in choline, phosphatidylcholine, and L- carnitine, and all of these are available in meat, fish, milk, and eggs [56]. TMA conversion into TMAO takes place in the liver by flavin mono-oxygenase-3 (FMO3) [57]. TMAO is associated with increased risks of ACVD [12,17]. Researchers have proved that when a choline-rich diet is fed to the mice ApoE−/− C57BL/6J, there is an enhancement in plasma levels of TMAO. The gut microflora remains intact in this case. Due to elevation in TMAO levels, there is macrophage foam cell formation and as result atherosclerotic plaques are also formed. On the contrary, the atherosclerotic plaques are reduced when germ-free mice are administered antibiotics to suppress gut microbiota. This is due to the elimination of plasma TMAO levels [58]. There is clear evidence that the circulating TMAO levels are responsible for increased thrombotic risks, including heart attack [59].

TMAO is also known to hinder reverse cholesterol transport in platelets. Some taxa of gut microbes are associated with increased levels of production of TMAO. These taxa include *Clostridiaceae* and *Peptostreptococcaceae* [17]. A structural analog of choline has been identified as 3,3-dimethyl-1- butanol (DMB), capable of reducing circulating TMAO. This DMB can be helpful in mitigating the ACVD [21,59]. TMAO also increases the platelet reactivity through stimulus-dependent calcium signaling. This increases the risk of thrombosis in people having elevated levels of

TMAO [60, 61]. TMAO is a potential therapeutic target for ACVD. Targeting FMO3 can also open broad avenues in the healthcare and research sectors.

Short chain fatty acids (SCFA)

Humans are unable to degrade some of the complex carbohydrates. The gut microflora mars these complex carbohydrates to produce short chain fatty acids (butyrate, acetates, and propionates) [28]. SCFA can be attributed to inflammatory processes [62,63]. SCFAs acts like a prominent source of energy for epithelial cells of the intestine. They have a vast role in immunity. They can enter the blood circulation and modulate the inflammatory responses. They can bind to G protein-coupled receptors (GPCRs), or they can also act by inhibiting histone deacetylases (HDACs) [62]. SCFAs, especially butyrate, are involved in host lipid metabolism, glucose homeostasis, and gut inflammation [63]. Butyrate produced by *Clostridia, Faecalibacterium prausnitzii,* and *Coprococcus catus* acts as an intestinal barrier. The butyrate also modulates intestinal macrophage function and contributes to decreasing colonic inflammatory processes [44]. Propionates lowers the immune response of the body. There is a decrease in the production of inflammatory markers like interleukin-13 and serum antibody immunoglobulin-E (IgE) in propionate treated animals for lung allergic airway inflammation [64]. Researchers have investigated that *Roseburia inulinivorans* that releases propionate, which helps protect against allergic airway inflammation [64]. Acetate contributes to lipogenesis, glycogenesis, and cholesterol synthesis [28]. Gut microflora thus acts as a conduit in the modulation of human immunity. This modulation of immune response by the SCFA's contributes to the progression of ACVD [22].

Bile acids (BA)

Gut microflora is involved in bile acid production and the ACVD progression [65]. Bile acids are produced by modifications of cholesterol in the liver in a series of reactions [66]. BA can act as a ligand for nuclear receptors and thus, modulate the signaling process. Studies conducted by the researchers show that BA has a binding action on the nuclear receptors Farnesoid-X-receptor (FXR) and G- protein-coupled receptor TGR5 to influence the metabolism of lipids and glucose [67, 68]. Primary BA plays a crucial role in the emulsification of fats and dietary lipids. After their synthesis, primary BA are released from the gall-bladder. The production of secondary BA depends on the microflora of the host organism. Dysbiosis of gut microflora often leads to the production of secondary BA. Gut microflora provides an edge over dihydroxylation, dehydrogenation and thus, converts primary BA into the secondary bile acids. Secondary BA can manipulate the amount of the hormones secreted from the gut [69, 70]. These secondary BA are responsible for the enhancement of the progression of ACVD [65].

Conclusions and future prospective

ACVD is one of the most fatal diseases of the world, and it was estimated that about 17.9 million people died in 2019 due to cardiovascular diseases. The gut microbial species produce certain bioactive metabolites, which can increase the chances of ACVD. It has been clearly shown that gut inhabitants are also responsible for different pathological conditions in recent years. With the advancement in technologies and a better understanding of gut microflora species, it is imperative that gut microflora targeted therapies can be used as an effective therapeutic strategy against ACVD. Further investigation of the mechanism of action of the gut microbial metabolites can contribute to the prevention of atherosclerotic disorders *in vivo*.

Those gut microflora species, which have an intense anti-inflammatory action, can delay the atherogenic plaques and counter inflammatory responses in the human gut. Atherogenic plaques are also an outcome of inflammation. Metabolites that can delay inflammation can be checked alternatively through different approaches and therapies might be prescribed to prevent gut dysbiosis. Probiotic therapies, administration of antibiotics, changes in diet can be looked upon for prevention of gut dysbiosis. Prebiotics like resveratrol, meldonium which are used profoundly can be modified according to the studies. Detailed studies can be performed to check for the abnormal amounts of microbial population in these pathologies. Better sampling methods can be introduced with time to check gut microfloral composition. This would ensure precise identification of gut dysbiosis and thus can prevent ACVD. These strategies would lead to a specificity in targeted prevention of ACVD by countering the inflammatory metabolites.

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Declaration

Authors declare no conflict of interest among themselves.

Author's Contribution

PG prepared Figures; PG drafted manuscript; PG, KB, NG, PL, and R. edited and revised manuscript; PG, KB, NG, PL, and R. approved the final version of the manuscript.

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