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Diabetogenic Microbiome - A Review on the Microbes Involve in Diabetes

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Abstract

Diabetes has emerged as a pandemic and has caused a great percentage of mortality all over the world according to the World Health Organization (WHO). This worldwide health issue has turned to be a major concern as it shares its role in the onset and progression of many other metabolic diseases along with it. Several factors that include microbes such as bacteria and viruses, diet, and lifestyle comes into play in the progression of this autoimmune disease. Studies done on humans and experimental animal models have provided a great deal of knowledge on how bacteria and viruses are involved in the pathogenesis of diabetes. Nevertheless, the need for more investigations is required to understand the relationship between the microbes and disease development. This review deals with the underlying reasons of diabetes Type I and Type II especially with respect to the microbes in the body and the subsequent changes caused through them.

Keywords: Diabetes, Disease, Enteroviruses, Gut microbiota, Immune system, Interferons

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Introduction

Scientists have been studying and conducting experiments to understand the causes of various health issues and ways to correct it. Researches were done on the diseases that pose a threat to the health. One such metabolic disease is diabetes, which have been affecting millions of people worldwide and has also turned out to be lethal in most of the cases. Moreover obesity and cardiovascular diseases are the most common health issue in today's date.

Human have been diagnosed with Type I diabetes, Type II diabetes as well as gestational diabetes mellitus during the early stages of pregnancy. Type I diabetes or Insulin dependent diabetes mellitus or Juvenile diabetes is an autoimmune disease as the T cells of the immune system causes impairment of the pancreatic β cells that produces insulin thereby resulting in insufficient insulin production. The cells being unable to uptake sugar is left to accumulate in the blood causing hyperglycemia. This Insulin dependent diabetes mellitus was found to affect more than 542000 children in 2015 as estimated by the International Diabetes Federation and is increasing by an annual rate of 3%.[1] Type II diabetes or non-insulin dependent diabetes is due to insulin resistance in the body, changes in the lipid profile and high blood pressure as well as insulin deficiency. Studies have found high levels of branched chain amino acids (BCAA), oxidative stress and aromatic amino acids (AAA) to be associated with insulin resistance development.[2] Moreover, pregnant women have been diagnosed with gestational diabetes mellitus (GDM) at the early stages of pregnancy. It might cause long-term effects like Type II diabetes, obesity to the affected woman as well the children.[3]

Diabetes is known to be both due to genetic and environmental factors. Archae, eubacteria, bacteria, and viruses have been found to contribute to the progression of diabetes as well as many metabolic diseases. Changes in the gut microbiome which is constitutively made up of archaea, eubacteria, bacteria; induction of autoimmune response against the β cells of pancreas by enteroviruses, Coxsackievirus B4 (CV-B4) have been elucidated in this review article.

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Gut Microbiota and Diabetes

Human gut is composed of many beneficial microorganisms' specifically different genera of bacteria which are collectively called microbiome. Studies have showed that about 500–1000 species of microorganisms prevailing in the human gut with about 100 trillion bacterial cells in the adult intestine.[1] These microbiota carries out fermentation, absorption of undigested carbohydrates, modulation of bowel motility, synthesis of some micronutrients and many other metabolic functions in the body.[4] Moreover, the gut microbiota are also found to interact with immune system for their proper development and functioning.

Imbalance in the gut microbiome can lead to many complications such as cardiovascular disease and obesity. This imbalance or dysbiosis of the gut occurs when the commensal relationship between the host and microorganisms is disrupted and also when there is quantitative change in the species of the gut microbiota.[5] The mucosal layer forms a protective barrier between the host's epithelial cells and the external microbes. Thus when there is dysbiosis of the gut, changes in gut permeability, sensory function as well as pain perception occurs through interactions

with epithelial cells and enteric nervous system that ultimately affects the intestinal mucosa.[6,7] When there is disruption in the gut mucosal layer, it might lead to metabolic endotoxaemia, inflammation, abnormal immunoregulation, and metabolic diseases.[8,9] Due to gut dysbiosis, the unstable microflora is believed to trigger a state of chronic low grade inflammation that makes the host prone to systemic exposure to lipopolysaccharide. This lipopolysaccharide binds to the TLR4 (Toll Like Receptors) and its co-receptors, which stimulates the release of pro-inflammatory molecules that disturbs the modulation of glucose and insulin metabolism.[7] In many studies, it was suggested that change in the gut microbiome contributes to the progression of Type I diabetes and Type II diabetes as well. Improper diet, hormones, macronutrient and micronutrient deficiency are among the many factors that caused gut microbiota dysbiosis. Thus, when there is alterations in the abundance, stability, and connectivity of gut microbiota, there is occurrence of Type I diabetes.[1]

Microbial Composition in Gut

The diversity and stability of microflora found in the gut carrying out metabolic functions are related to gut as well as of the immune system. Studies have found that people who are not diabetic, normally contains 60–80% of *Firmicutes*, 20–30% of *Bacteroidetes,* approximately 10% *Actinobacteria* and *Proteobacteria,* and *Escherichia* in the least amount.[4] In the gut of humans the genera, namely, *Akkermansia*, *Prevotella, Blautia, Ruminococcus, Escherechia, Lactobacillus, Megasphaera, Sutterella,* and *Acidaminococcus* are found. Moreover, genus *Faecalibacterium, Phascolarctobacterium, Dorea, Parabacteroides, Clostridium, Butyricicoccus, Roseburia, Lachnospira, Dialister, Oscillospira, Coprococcus,* and *Haemophilus* are seen in the gut. Among these *Bacteroidetes*, *Dialister*, *Campylobacteria, Enterococcus, Butyricimonas, Odoribacter, Rickenellaceae, Sutterella,* and *Atopobium* are found to be the taxonomic biomarkers for diabetes. While *Gemmigera, Bifidobacterium, Clostridiales*, *Ruminococcus, Oscillospira, Lachnospiraceae,* and *Veillonellaceae* were found to be the biomarkers of normal non-diabetic people.[3]

Through their experiments researchers have found that in the newly diagnosed diabetic patients the concentrations of *Firmicutes (Lactobacillus)* and *Proteobacteria* were high and low concentrations of *Bacteroidetes* and *Verrucomicrobia* while the concentration of *Actinobacteria* was found to be low in the already known diabetic patients. When compared to non-diabetic patients, the concentration of genus *Akkermansia, Blautia and Ruminococcus* in newly diagnosed diabetic patients was low. However, while in the known diabetic patients, the concentration of *Megasphaera, Escherechia, Acidaminococcus and Akkermansia* was found to be higher than the non-diabetic patients with *Sutterella* genus in low concentration in the already known diabetic patients.[2]

In pregnant women, there was high abundance of *Bacteroidetes* and *Proteobacteria* and low abundance of *Firmicutes* and *Actinobacteria*.[3] Moreover, people affected with Type I diabetes had more amount of *Lactobacillus gasseri*, *Streptococcus mutans,* and *Clostridium sp* and less amount of *Roseburia intestinalis, Faecalibacterium prausnitzii*. In healthy children *Lactobacillus, Bifidobacterium, Blautia, Eubacterium rectale, Prevotella* are generally found in higher amounts thereby keeping the gut epithelium intact. But in contrast to the healthy children, the diabetes affected children had more amounts of *Clostridium, Bacteroidetes* and *Veillonella*.[4] According to a study done on non-diabetic people, it was suggested that *Clostridium sp, Eubacterium rectal, Faecalibacterium prausnitzii,* and *Roseburia intestinalis, Roseburia inulinivorans* must be in high concentration. While the Type II diabetic patients must have *Bacteroides coccae, Clostridium hathewayi, Clostridium ramossum, Clostridium symbiosum, Eggerthella sp, E. coli, Desulfovibrio sp* in more amounts.

Gram negative *Bacteroidetes* and *Proteobacteria* might cause Type II diabetes development through an endotoxin-induced inflammatory response as the endotoxin, lipopolysaccharide exists in high concentration as a main outer cell membrane component.[10] Type II patients had more *Firmicutes* especially *Clostridium sp*, *Bacilli* (*Lactobacillus*) while *Bacteroidetes* were in less amount thereby resulting in higher ratio of *Firmicutes* to *Bacteroidetes.* While in another study it was found that diabetic patients had more concentration of *Bacteroidetes* while low concentration of *Firmicutes.* Thus these studies prove that composition of bacteria in gut of Type II patients differs to that of healthy people.[11] Although most of the studies found *Bifidobacterium, Bacteroides, Faecalibacterium, Akkermansia,* and *Roseburia* to be negatively associated with T2D while *Ruminococcus, Fusobacterium,* and *Blautia* were directly associated with T2D but variation was seen in the quantitative results of microbe study. This variability observed in the results of microbiome literature was thought to be maybe due to several factors like different geographical locations, food habits, genetics, medications.[12] The bacterial phylum and their concentration present in the pre diabetic, newly diagnosed diabetic and known diabetic patients are listed in the Table 1.

In an experiment carried out by transferring gut microbes from MyD88-/-NOD mice as well as MyD88-/-B6 mice to NOD mice showed more prevalence of *Bacteroidetes*, *Actinobacteria, Lachnospiraceae* with less amount of *Firmicutes*, *Ruminococcus*, and *Lactobacillaceae* in gut of NOD mice as compared to the mice that received exogenous microbes from MyD88-/-B6 mice. This suggested that through the transfer of exogenous microflora to the previously unstable gut, the microflora composition can be corrected. As a result this induced a proper working immune system that delayed the onset of diabetes in the recipients.[13] In many studies, it was found that lactate and butyrate deficiency might be a trigger for diabetes. Butyrate which helps in the growth of beneficial bacteria and production of IL-10 is produced by the bacteria belonging from the Firmicutes phylum mostly *Bifidobacterium spp*. and *Faecalibacterium prausnitzii. Bifidobacterium spp* has been found to have anti-inflammatory effects through the production of GLP2 and also reduces the intestinal permeability thereby providing protection from influx of harmful microbes and their by product. Thus less abundance of lactate and butyrate producing bacteria may give rise to anti-inflammatory effects and autoantibody status along with islet destruction.[6,8,14-16]

Table 1: Taxonomic distribution of bacterial phylum and their concentration present in the pre diabetic, newly diagnosed diabetic, and known diabetic patients according to the studies done by Gaike *et al.* (2020)

Phylum	*Pre diabetic patients (%)*	*New diabetic patients (%)*	*Known diabetic patients (%)*
Bacteroidetes	61.9	41.31	13.8
Firmicutes	22.2	38.4	55.1
Proteobacteria	8.1	16.14	20
Actinobacteria	3.4	2.6	6.8

Gut Microbiome Dysbiosis Affecting Immune System

In a healthy individual, the microbiome regulates the immune system through intestinal epithelial cells.[6] Studies have shown that more diverse the gut microflora is less is the onset of diabetes.[13] Endotoxins increases the level of pro-inflammatory cytokines and thereby causes impairment of pancreatic β cell function. This ultimately leads to the development of diabetes. Dysbiosis of gut microflora causes destruction of intestinal mucosal barrier that simulates the efflux of lipopolysaccharide (LPS) or endotoxins as well as fatty acids. This is followed by activation of toll like receptors (TLR4) causing metabolic inflammation. An experiment was conducted to test if TLR4 and Type I diabetes are correlated. They further concluded that the interaction between gut microbiota and innate immune system induces the development of Type I diabetes.[1]

Viral Pathogenesis in Diabetes

In Type 1 diabetes, the CD4+ and CD8+ T cells recognizes the β cells as antigens and destroys them. Viral infection occurs through two mechanisms simultaneously – Molecular mimicry and Bystander mechanism. In molecular mimicry, the antiviral as well as the autoreactive T cells gets activated as a result of presentation of viral antigens by Antigen Presenting Cells (APC). The P2C protein sequence of CVB4 virus was found to partially match with human GAD (Glutamic Acid Decarboxylase), biosynthesizing enzyme of GABA. Although cross reactivity between P2C and GAD was proposed to explain the capacity of CVB4 to induce diabetes, but studies shows that without other diabetes susceptibility factors and less homology between the viral and β cells antigen, diabetes development does not occur. Thus molecular mimicry only acts as the preliminary step for onset of diabetes by enhancing the autoimmune responses. Host specific immune responses are generated when there are sequence and epitope homologies between viral antigens and host determinants. In Bystander mechanism, viral infection induces inflammation that destroys tissues and releases sequestered islet antigens. The autoreactive T lymphocytes are stimulated which are directed against these sequestered antigens, thereby giving rise to disease development. Researchers found that β cell destruction is caused by systemic production of interferons (IFNγ) as well as Type 1 and Type 2 cytokines through unmasking of β cells.[17-19] Studies have showed that diabetic patients are more at risk of enteroviral infections. Enterovirus is small viruses which are devoid of envelope but has an icosahedral capsid. These viruses are single stranded, linear, non-fragmented positive RNA which is capped by the viral protein genome linked (VPg) protein in the 5′ end. These enteroviruses are from the Picornaviridae family that includes the genera, namely, *Rhinovirus, Hepatovirus, Parechovirus, Cardiovirus, Kobuvirus, Aphtovirus, Erbovirus,* and *Teschovirus.* Human enteroviruses are of five subgenera which are *Poliovirus (PV); Coxsackievirus* (CV-A), CV- B*; Echovirus* (E-V); and unclassified enterovirus. Enteroviruses have been isolated from the pancreas of diabetic patients and were found to contain anti-CV antibodies in their bodies. In diabetes pathogenesis, enteroviruses are believed to damage β cells and releases interferons, antigens and includes both innate and adaptive immune system.[20] Several strains of *Coxsackievirus* such as CV-B4, CV-B4E2, and CV-B5 of which CV-B specially CV-B4 were found to be most frequently involved. Of these strains CV-B4E2 provokes hyperglycemia. Type I diabetic patients were found to host CV-B4 RNA which is homologous to that of CV-B4E2 and CV-B4 VD2921 in their peripheral blood mononuclear cells (PBMC).[19] Interferon alpha (IFN α), a marker of viral infection is found to be present in high concentration in the plasma of CV-B infected Type I diabetic patients.[21] CVB4 infection induces inflammation which is mediated by natural killer cells. There is a possibility that enteroviruses might target the β cells through surface molecules such as integrin, $\alpha_v\beta_3$, and poliovirus receptors which are found expressed on human β cells. Viruses may unmask β cells to facilitate recognition by CD8+ T cells. Unmasking of β cells by interferon production and upregulation of MHC (Major Histocompatibility Complex) Class I molecules on β cells, thus might lead to autoimmune attack.[17] The presence of IFN α, enterovirus RNA (specially CV-B4 RNA) as well as anti enteroviral antibodies are directly correlated to Type I diabetes. Several studies were carried out on the CV-B4 infection of pancreatic cells that indicated that the pathogenesis of diabetes by viral strains CV-B4E2, CV-B4 VD2921, including the prototypes of CV-B2, -B3, -B4, and -B5 serotypes are prone to infect and cause damage to the human β cells *in vitro*.[21-23] Enterovirus particularly CV-B4 were found to stimulate the expression of MHC molecules that in turn induces the autoimmune response against β cells.[24] Moreover, according to a study CV-B5 and CV-B induced cytokines (IL-1β and IFN α) triggers the expression of chemokines such as IL-15 and ICAM-1 that ultimately give rise to the activation of mononuclear cells in islets of pancreas which takes part in the early infection process.[25] Enteroviral infections destroys pancreatic β cells indirectly by causing inflammation of the endocrine pancreas through production of nitric oxide radicals, cytokines and other toxins.[26] These β cells, post infection, may be also destroyed by continuous infection because of amplication of the viral genome, prolonged infection which may result in persistent activation of dendritic cells that is coupled with long-term presentation of viral antigens as well as autoantigens to the T lymphocytes. In addition, a subsequent initiation of an antiviral and autoimmune response provokes damage of β cells.[27]

At the time of Picornavirus infection (particularly encephalomyocarditis virus, CV-B4), interferons mainly IFN α. IFN β and IFN γ are produced in the early stages of infection. These interferons are found to induce an antiviral state in IFN-responsive target cells which results in the protection of target cells from infection. When there is persistent increase in the concentration of type I IFNs (IFN α. IFN β), it leads to the damage and death of β cells, thereby reducing insulin biosynthesis or by activation of self-reactive T cells.[18,28]

During viral infections, interferons, interleukins, and tumor necrosis factors are found to be released in high amounts which causes tissue inflammation by regulating the expression of β cell antigens. *In vitro* studies have reported that reovirus causes direct upregulation of HLA Class 1 molecules in islets, thereby inducing islet autoantibodies. Measles and mumps virus stimulated IL-1 and IL-6 production and increases the expression of Class 1 and Class 2 antigen as well.[18,29]

Among all the virus causing insulin resistance thereby leading to development of diabetes, Lymphocytic choriomeningitis virus (LCMV) is also found to infect humans, but in rare cases. It is usually a murine virus causing pathogenesis of diabetes. Subjects which had defective interferon response in β cells caused by the expression of suppressor of cytokines signaling-1 (SOCS-1) in the β cell compartment, were found to be more susceptible to viral infections that aids in the progression of the metabolic disease diabetes.[30]

Conclusion

Type I diabetes, Type II diabetes, and Gestational *Diabetes mellitus* have emerged as the burning concern internationally. With the advances in science, numerous studies are being conducted to understand the causes of this metabolic disorder. Imbalance in the gut microbiome, sedentary life, improper food habits as well as hormones are some of the factors that leads to the onset of diabetes. Bacteria and viruses are found to be associated with the progression of diabetes. However, some topics like the underlying mechanism(s) by which the bacterial flora and CV-B3 causes lysis of the pancreatic cells leading to the onset and progression of diabetes, exact contribution of the gut microbiota and their products on the pathogenesis of diabetes, fluctuation of the gut microbiota is yet to be elucidated.

Future Scope

Diabetes being one the critical metabolic disorder, it is utmost required to know its various causative factors. The recent research about various factors involve in diabetes showed that microbes are playing a crucial role as a causative agent. This paper critically reviewed the microbial agents and their involvement in causing diabetes. Hence, this review article will help the researchers to deeply explore the microbial connection in diabetes.

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