Review Article

Early life antibiotic exposure and inflammatory bowel disease

Rashmi Agrawal[1], Amit Agrawal[2], Janice Jacson Mandumpala[3]

From [1]Consultant Gynaecologist, Department of Obstetrics and Gynaecology, Devmata Hospital, Bhopal, Madhya Pradesh, India, [2]Associate Professor, Department of Pediatrics, Gandhi Medical College and Kamla Nehru Hospital, Bhopal, Madhya Pradesh, India, [3]Pharm D Intern, Department of Pharmacy Practice, Nirmala College of Pharmacy, Kerala University of Health Sciences, Cochin, Kerala, India

ABSTRACT

Inflammatory bowel disease (IBD) is known to affect millions of people worldwide and is primarily caused due to abnormal due to abnormal immune responses to intestinal microbes. IBD is primarily categorized under two main types – ulcerative colitis and Crohn's disease. Although the cause of IBD is still an unsolved puzzle, its development can be attributed to the presence of various risk factors including antibiotic use. The intestinal immune system is the point of genesis of IBD and anything that creates an imbalance can trigger IBD. Alterations in the gut microbiota can lead to reduced microbial populations and lead a chronic inflammation state. Antibiotics may influence microbial maturity and can hamper the formation of a strong immune system. This makes an individual vulnerable to the threat of infections and subsequent development of a chronic inflammation state. Studies have implicated that the use of antibiotics during pregnancy, antepartum, and among neonates is associated with the development of IBD. Adequate development of the microbial niche takes place within the first 2 years of life and extensive administration of antibiotics during this period can pose a significant risk for the development of IBD. Animal studies have also documented the role of *in utero* antibiotic exposure during the antepartum period and the subsequent risk of developing IBD in the offspring. This review summarizes the role of antibiotic exposure during pregnancy and infancy in the development of IBD.

Key words: Antibiotics, Crohn's disease, Infants, Inflammatory bowel disease, Neonates, Pregnancy, Ulcerative colitis

The intestinal microflora is a crucial regulator of the gut immune system. It is also a mediator of various chronic diseases affecting the gut such as inflammatory bowel disease (IBD) and coeliac diseases [1]. The gut microbiome is established early in life. The primary activities of this gut microbiome are nutritive, metabolic, immunological, and protective functions. Although the establishment of the gut microbiome is believed to be a part of ecological succession, recent research has suggested the role of microbe-host interactions, and external and internal factors [2]. The normal gut flora acts as an effective barrier against colonization by various pathogenic organisms. This suppression and protective effect of the gut microflora is termed colonization resistance. Antibiotics are one such source of intruders that cause disturbances between the host and the normal intestinal microflora [3]. The extent of effect depends on the characteristics of the antibiotic used (absorption, route of administration, dose, duration, pharmacokinetic, pharmacodynamics profile, and spectrum of the agent), the time of exposure (prenatal, neonatal, and infant), and frequency of exposure. The association between antibiotic use and the development of IBD needs further scrutiny and discussion among the members of healthcare [4].

Access this article online	
Received - 22 November 2022 Initial Review - 26 November 2022 Accepted - 28 November 2022	Quick Response code
DOI: 10.32677/ijch.v9i11.3741	

INTESTINAL MICROFLORA: COMPOSITION AND DEVELOPMENT

The intestinal microflora harbors around 400 microorganisms and most of them are bacteria [5]. In healthy humans, these microflorae are present at the distal ileum and colonic region. The predominant species in these regions are bifidobacteria, eubacteria, clostridia, and lactobacilli enterococci and coliforms [6]. There are four different sites or microhabitats for the intestinal microorganisms and they are the surface of the epithelium, the crypts, the mucosal gel layer, and the intestinal lumen [7]. There is always competition within the bacterial ecosystem to occupy the various adhesion sites and the microbiota most suitable for the host is established by the principle of natural selection [8].

The normal microbiome is the first line of defense that competes with the invading organisms and directly interferes with the settlement of the newly arrived organisms [9]. The

Correspondence to: Dr. Amit Agrawal, Associate Professor, H. No – 28, Ravidas Nagar, Opposite Nizamuddin Colony, Indrapuri, Bhopal, Madhya Pradesh, India. E-mail: agrawaldramit@yahoo.co.in

normal microbiome influences the development of the humoral immune response, modulates the host immune response to various pathogens and antigens, and simultaneously controls the onset of hypersensitivity reactions. Therefore, the gut-associated lymphoid tissues have a crucial role in identifying and targeting pathogenic and non-pathogenic organisms with the subsequent development of immune response or tolerance [10-13].

The establishment of the climax intestinal microbiome is an outcome of various factors influencing its development. The key hos-related factors include the anatomical development of the intestinal tract, intestinal pH, the chemical profile of the bile acids, microbial interactions, availability of mucosal receptors, and exposure to various drug therapy [14]. The fetal intestine is exposed continuously to the amniotic fluid and remains sterile. Post-delivery the fetal intestine is challenged with various antigens. Microbes from the maternal vaginal and perineal area enter the mouth and subsequently the gastrointestinal tract of the infants born through the vaginal route. Within minutes after the delivery, the infant's intestinal gut is occupied by the cervical flora of the mother [15,16]. The maternal intestinal flora is also an ideal source of bacteria for the newborn. However, the transmission rates are much lower and the infants also acquire microbes from environmental exposure [17].

The further multiplication of the bacteria and development of the infant gut microbiome is influenced by the host enteric environment, microbial interactions, and the infant diet provided. On the 1st day of life, enterobacteria, streptococci, enterococci, and staphylococci have already colonized [18]. As the infant is exposed to amounts of oxygen, these aerobic organisms consume the oxygen and reduce the oxidation-reduction potential of the intestinal environment. Consequently, a favorable environment is created for the settlement of a more diverse population of microbes, including anaerobic organisms [19]. A summary of the organisms in each stage of development through breastfed and formula-fed infants is depicted in Table 1.

There are various factors affecting microbial presence and succession in a newborn. The mode of delivery whether vaginal or cesarean section plays a crucial role in exposing the newborn to various microbes. Preterm birth, antimicrobial exposure, and hygienic conditions influence the microbial load of the newborn. Similarly, in developed countries, strict hygienic practices to avoid the spread of bacteria in maternal and neonatal wards have influenced the colonization patterns in newborns [24,25]. In developing countries, newborns are exposed to higher bacterial load [26]. In a neonate, the use of antibiotics has a strong influence on the development of the intestinal microbial colonies. The emergence of bacterial resistance is quite common among neonates treated in the neonatal intensive care unit [27]. Therefore, it is particularly important to study and be aware of the various factors that influence the microbiome, particularly, antibiotic exposure [2].

MATERNAL ANTIBIOTIC EXPOSURE

In a cohort study conducted by Anne *et al.* among children in Sweden, it was observed that children exposed to antibiotics during pregnancy are at high risk for IBD (particularly very early onset IBD) regardless of gastroenteritis, in comparison to the general population controls [28,29].

Although direct evidence is lacking, certain animal-based studies have highlighted that peripartum exposure to antibiotics is associated with an increased risk of developing IBD. Miyoshi *et al.* conducted a study among dams to examine the temporal impact of often-used cefoperazone on both offspring and maternal microbiota when administered during the peripartum period. It was observed that cefoperazone-exposed offspring developed persistent gut dysbiosis while progressing to adulthood [30]. Thus, early life exposure to antibiotic-induced maternal dysbiosis can elicit a high risk of IBD among offspring. This effect is particularly pronounced during the critical window period for gut microbiome assemblage and immune system programming. Similarly, Peris *et al.* examined the role of antepartum antibiotic drug therapy among pregnant mice in increased susceptibility of the offspring to experimental colitis through alteration of the gut microbiota. On treatment of pregnant mice with cefazolin 6 days before the due date, it was observed that antepartum antibiotic therapy influenced the offspring's fecal bacterial composition and, hence, the bacterial functional pathways before the onset of colitis. Therefore, this study is in supports the hypothesis that antepartum antibiotic therapy can modulate the offspring's intestinal bacterial colonization and may lead to IBD [31]. Anjelique *et al.* have demonstrated that the transfer of antibiotic-perturbed microbiota from mothers to offspring could affect their risk of developing IBD. As per the findings of their study germ-free adult pregnant mice inoculated with a gut microbial community shaped by antibiotic exposure transmitted their perturbed microbiota to their offspring with high fidelity, and they eventually developed marked colitis [32]. Taken together,

Table 1: Intestinal microflora in breast-fed and formula-fed infants [20-23]

Time elapsed post-delivery	Breast-fed infants	Formula-fed infants
Day 1	Predominantly enterobacteria	Predominantly enterobacteria
Day 6	Bifidobacteria > Enterobacteria but the former is not the predominant species	Levels of enterobacteria remain stable, Bifidobacteria levels increase slowly 1/10th of breastfed infants
1–2 months	Bifidobacteria > Enterobacteria (1000:1), the former is the predominant species and other organisms remain suppressed	End of 2nd month Bifidobacteria increases with the simultaneous growth of Enterobacteria, Enterococci, Lactobacilli, Clostridia, and Bacteroides
End of 3 months	Bifidobacteria decreases, and bifidus flora only seen in 50% of the population	Bifidobacteria increases gradually but much lesser extent than breastfed infants

these animal-based studies indicate that antibiotic exposure shaping the maternal gut microbiota has effects that extend to the offspring, leading to long-term consequences.

Another area of concern is the use of tumor necrosis factor-alpha inhibitors among pregnant women. These inhibitors are most often transferred across the placenta and can increase pediatric susceptibility to infections. Although the clinical significance of the relatively high frequency of infections and antibiotic use among offspring is questionable, it can be reassured that the fetus is at high risk for developing infections due to the immune-compromising nature of these inhibitors [33,34].

Surprisingly, in a population-based analysis conducted by Munyaka *et al*. has observed that maternal infections that would warrant antibiotic use antepartum or peripartum do not affect the risk of developing IBD in the offspring [31]. Therefore, there are support and contradictions to the theory of peripartum and antepartum maternal antibiotic exposure to the development of IBD in children. Hence, long-term future studies are warranted to causally assess the risk of antibiotic use.

PERINATAL AND INFANT ANTIBIOTIC EXPOSURE

The use of antibiotics is higher among infants born through cesarean section and among those who are preterm in comparison to those born vaginally. Preterm infants depict aberrant gut microbial colonization that affects the maturation of the immune system [35]. Antibiotics directly perturb the intestinal microflora, disturbing the microbial environment both in children and adults [36]. The infant microbiota undergoes drastic changes during the first 2 years of life and is crucial in establishing a stable gut environment. Fiona *et al.* have observed that on administration of parenteral ampicillin and gentamycin among infants led to higher proportions of *Proteobacteria* and lower proportions of *Actinobacteria*, *Bifidobacterium* and *Lactobacillus*. The altered gut microbiome among infants is a concern given the health-beneficial effects of *Bifidobacterium* and *Lactobacillus* (mainly employed in probiotics) and the pathogenic nature of *Proteobacteria* in a favorable environment [37].

Another consequence of early antibiotic use is the delayed maturation of the intestinal microbial community relative to unexposed children. This is achieved by the suppression of early-life biomarkers such as *Lachnospiraceae, Enterobacteriaceae, Erysipelotrichaceae*, and numerous predicted gene pathways, thus causing the gut microbiome of the antibiotic-exposed infants to appear less developed than those unexposed [38]. *Lachnospiraceae* is highly abundant in the digestive tracts of humans and rarely thrives elsewhere. They often engage in the production of short-chain fatty acids and butyrate molecules, and regulation of host immune response through colonic regulatory T-cells and macrophages [39-41]. Therefore, the gut microbiome has a significant role in the development of immune tolerance.

Although there is strong evidence documenting the association between increasing antibiotic use in the first few years of life and childhood-onset IBD, the risk is higher with antibiotic use in the first 6 months of life [42]. Identically, Shaw *et al.* found that 58% of the cases diagnosed with IBD (mean age of onset 8.4 years), predominantly Crohn's disease, had ≥1 antibiotic exposure in their 1st year of life, as opposed to 39% of the controls [43]. Kronman *et al.* also found that antibiotic exposure before the age of 1 year is associated with developing IBD with a hazard ratio of 5.5 in comparison to those exposed before the age of 5 and 15 years with a hazard ratio of 2.6 and 1.6, respectively. Furthermore, they observed that each antibiotic course increased the risk of IBD by 6% and a dose-response effect also existed with those receiving >2 antibiotics carrying a higher risk in comparison to those receiving two courses [44].

Scientists have also elaborated on the role of the epigenetic landscape in modulating the course of IBD among those exposed to antibiotics. Antibiotics have the potential to induce epigenetic changes and consequently change the expression of immune cells or mRNA [45-47] (Table 2).

Table 2: Impact of antibiotics on the epigenome in intestinal inflammation

Antibiotic	Epigenetic mechanism	Tissue/ cells	Mechanism	Model	Reference
Isotretinoin	miR	T-cells	3 miR overexpressed in naive T-cells and potentially downregulate 777 miR targets	BALB/c mice	Becker *et al*. [45]
Metronidazole	miR	T-cells	5 miR were significantly lower in naive T-cells resulting in the prediction of 340 potentially upregulated miR targets associated with IL-2 activation and signaling, cytoskeleton remodeling and epithelial-to-mesenchymal-transition (EMT).	BALB/c mice	Becker *et al*. [45]
Doxycycline	miR-144-3p	T-cells	Overexpression of miR-144-3p that resulted in the prediction of 493 potentially downregulated miR targets involved in protein kinase A (PKA), protein kinase B and nuclear factor of activated T-cells (NFAT) signaling pathways	BALB/c mice	Becker *et al*. [45]
Tetracyclines	miR-150, miR-155, miR-375 and miR-146	Colonic tissues	Reduce miR-150 and miR-155 expression, upregulate miR-375 and miR-142	DSS-induced colitis in mice and bone marrow-derived macrophages	Garrido *et al*. [46]

miR: Micro RNA, Balb C: Bagg and albino mouse model, DSS: Dextran sulfate sodium

CONCLUSION

Antibiotic use does have a role in the onset of IBD. Early life exposure to antibiotics can disturb the microbial colonization in infants and can subsequently lead to disturbance in the gut immune system. Both maternal and infant exposure to antibiotics serve as potential risk factors for IBD. The findings from this review if confirmed with long-term prospective studies can prevent the onset of dysbiosis-related chronic inflammatory conditions such as IBD.

AUTHORS' CONTRIBUTIONS

RA: Concept, interpretation of data and data analysis, drafting the article, literature review, and revising the article critically for important intellectual content. AA, JJM: Acquisition and interpretation of data, data analysis, drafting the article, and literature review; all the authors approved the final manuscript.

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Funding: None; Conflicts of Interest: None Stated.

How to cite this article: Agrawal R, Agrawal A, Mandumpala JJ. Early life antibiotic exposure and inflammatory bowel disease. Indian J Child Health. 2022; 9(11):195-199.