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Review Article

A review on COVID-19, colonising microflora and microbial links to age-related differences and off-target effect of live vaccines like BCG

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ABSTRACT

Age-related expression for a disease is well known. The applicability of such an expression for SARS-CoV-2 prompted this review. Whenever an infection is highly prevalent, the younger age groups get more affected. But this is not seen in COVID-19. The severity of COVID-19 disease is more and sometimes fatal in adults when compared with children and found to be less severe. This shows a striking difference as generally children tend to get more affected with most of the respiratory viruses.

Can this be explained by the differences that are observed in their oro-pharyngeal, lung, nasopharyngeal and gastrointestinal microbiota? This review addresses the potential of resident microbiota for the spectrum of expressions in susceptible population through various mechanisms. In the nasopharynx, where microbial interactions and competition may limit the growth of SARS-CoV-2, children are more extensively colonized with viruses and bacteria than adults. One study found no discernible differences in the nasopharyngeal microbiota between SARS-CoV-2 patients and healthy individuals, whereas other investigations found significant differences in the oro-pharyngeal, lung and gut microbiota between these groups.

There is a reduced load of bacteria in the gut microbiota of the patients who are infected with COVID-19; especially the bacterial phyla such as *Faecalibacterium* are found to be very less in the gut but there is relatively a higher load of other organisms such as *Bacteroides.* It is known that *Faecalibacterium* have a lot of anti-inflammatory properties and *Bacteroides* show decreased gastrointestinal ACE-2 expression. The microbiota in the human gastrointestinal tract differs with age. Children's guts exhibit higher concentrations of *Bifidobacterium.* These variations in the gut microbiota of patients have also been noted between those who do and those who don't excrete SARS-CoV-2 in their feces. However, these results, which are based on the gut flora of each patient, may be affected by factors like food, age, use of antibiotics and their immune system. This relation between the gut microbiota and the severity of COVID-19 disease in patients is studied and it is still unclear. Randomized control trials (RCT) of BCG are being conducted to lessen the severity of COVID-19. Oral polio vaccination and the measles-containing vaccine (MCV), in addition to BCG, have been proposed as potential factors in the difference in COVID-19 severity. To lessen the severity of COVID-19, a randomized control trial of the MMR vaccine has been planned. Understanding the mechanism underlying the age-related variations in COVID-19 severity through the colonizing microbial flora and off-target effects of live vaccines (BCG, etc.) would provide important cognizance and open up many opportunities for the management and cure of this novel infection.

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1. Introduction

1.1. COVID-19 virus

SARS Coronavirus-2, a member of the Coronaviridae family, is the cause of COVID-19 disease. The virus was initially discovered in China and quickly spread throughout the entire globe. The disease ranging from mild common cold to fatally severe illness leads to death. Comparing SARS Coronavirus-2 to other Coronaviruses like SARS and MERS, it is less pathogenic but more contagious. The SARS Coronavirus-2 has affected and spread across the globe, therefore the WHO declared it a pandemic on March 11, 2020. The novel SARS-CoV-2, which causes the disease COVID-19, has spread quickly throughout the world. A few studies indicate that children and adults are equally susceptible to SARS-CoV-2 infection.[1]

However, more recent research indicates that children would be less likely to contract the virus after coming into touch with SARS-CoV-2 positive person.[2–6] Individuals with symptoms and those without symptoms could display a similar viral load.[7–9] However, it is noted that transmission in schools between children and either other children or adults is unusual.[10–12]

Compared to SARS-CoV-1 and the Middle East Respiratory Syndrome(MERS-CoV), children are less likely to be infected with SARS-CoV-2 and exhibit very few symptoms.[13–15] The severity and prevalence of infections brought on by the majority of respiratory viruses such as metapneumovirus, respiratory syncytial virus(RSV), influenza viruses or parainfluenza viruses are both higher in children than they are in adults. However, this pattern is noticeably different.[16]

SARS Coronavirus-2 is a single stranded positive sense RNA genome. It is about 30kilobytes in size. It is an enveloped virus and spherical in shape with some degree of pleomorphism. This virus is composed of 4 structural genes, namely, membrane (M), spike (S), envelope (E) and nucleocapsid (N) and also 15 non-structural proteins. Phylogenetic studies revealed SARS Coronavirus-2 has 89% similarity to Bat Coronaviruses and 80% similarity with SARS Coronavirus-1. The clinical symptoms include fever, cough, myalgia or fatigue, headache, hemoptysis and diarrhea. The incubation period in humans ranges between 3 and 6 days.

Children and young adults are less severely affected by the illness but this infection causes higher complications in adults, especially those with comorbidities.

Currently, reverse transcriptase polymerase chain reaction is used to diagnose Coronavirus (RT-PCR). Almost all the government and private organizations started screening COVID-19 by RT-PCR. The number of

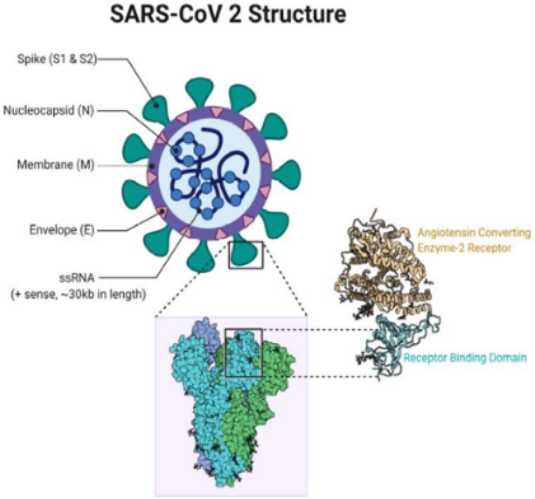

Fig. 1: Structure of the virus relevant to pathogenesis Ref.https://commons.wikimedia.org/wiki/File:Struktura_SARS-CoV_2.jpg

infections has come down when compared to the initial stage. Recently, COVID vaccines have also been introduced in different countries globally especially for health care workers. Also, different types of vaccines are under clinical trials. At this point of time, we must look for the immune response elicited by the host to these vaccines after being administered. Several immunological studies are undergone to know about the immune response for COVID-19 infection. But still there is a lack of information such as kinetics of total antibody response, neutralizing antibody response, the effect of secondary or re-infection with new variants of SARS Coronaviruses and so on. Studies can be taken up for monitoring the immune response in naturally infected, vaccinated and asymptomatically infected people based on the detection of IgG antibody specific to SARS Coronavirus-2.

1.2. Global scenario

According to WHO, globally, more than 100 million cases of SARS Coronavirus-2 were reported and over 2.2 million deaths have also occurred so far. Almost all the countries and continents have been affected by SARS Coronavirus-2. Initially, China was the country which reported significant number of cases and deaths. Later it spread rapidly all over the other countries; more than 210 countries have been affected now. Italy reported increased number of deaths compared to other countries affected. After all the situation, recently the USA has been affected the greatest with more cases and deaths followed by the United Kingdom, India, Brazil, Russia, France and Spain. In the year 2021, a decreasing trend was observed. Globally, the vaccine was also introduced into health care settings and most of the countries are actively participating into it. Currently two vaccines are available for public

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use such as AstraZeneca/Oxford COVID-19 vaccine and Pfizer COVID-19 vaccine. Researchers are about to study the effect and duration of vaccine response. Similarly, studies are going on to evaluate the immune response and seroprevalence of COVID-19 disease among patients and healthcare workers. Seroprevalence data is significantly useful for vaccine management. A study from Denmark reported the seroprevalence of SARS Coronavirus-2 was 4% among healthcare workers.[17] Similarly, another study reported very low seroprevalence of SARS Coronavirus (about 1.9%) among blood donors.[18] These values are comparatively low when compared to other studies. The most effective way to assess IgG response is by ELISA. According to Gomez- Ochoa SA et al., 2020, systematic and meta-analysis were used to evaluate the incidence of SARS Coronavirus-2 in healthcare personnel. The prevalence based on RT-PCR testing was 11% and based on IgG antibody detection was 7%.[19]

1.3. Indian scenario

In India, COVID-19 infection has infected more than 10million people so far and more than 1,50,000 people have passed away as a result. The first case was reported in Kerala which originated from China. Later, it spread rapidly through person-to-person transmission and also due to travelers from all around the world who visited India. India is the leading cause of COVID-19 infection among all Asian countries and also the second highest leading cases of COVID-19 globally. The number of cases has started to come down from 2021. The first vaccination against COVID-19 also started on January 16, 2021. Emergency use of Covishield was approved by Drug Controller General of India on 1^{st} January, 2021. It is a viral vector-based vaccine developed by the University of Oxford. Another vaccine has also been available for public use from 2nd January, 2021 for emergency purpose called Covaxin, developed by Bharat Biotech, ICMR and NIV, India. The vaccine response is still not known. Studies are going on to identify the immune status and sero-conversion rate. A study from Pune, reported that the development of IgG response was 100 percent on 4^{th} week of infection and the antibody levels were higher in severely infected patients. It was reported that the seroprevalence of COVID-19 antibody among health care personnel in Mumbai was 19% (47 out of 244 numbers studied).

A nationwide household survey of 70 districts in India was conducted to identify the seroprevalence from the community.[20] This study reported the seroprevalence of COVID-19 IgG antibodies in individuals aged 10years or older was 6.6%. Another cross-sectional sero survey in Delhi reported that the seroprevalence was 25.42% as on October 2020. The different geographical regions may show a varied seroprevalence levels. Hence, the seroprevalence study is necessary to be conducted to know the impact of COVID vaccines in districts of India. Also, there is a lack of information on the duration of the immunity or protective vaccine response.

1.4. Intensity of viral exposure

It can be hypothesized that the severity of Corona infection may be influenced by the viral load to which a child is exposed or the intensity of the host tissue reaction to the virion. The extent of exposure in adults may be directly related to their need to be in many places as dictated by their job, travel and exposure in their work place and nosocomial exposure for health care workers. The world of children is limited to their home, schools or play areas and they occasionally travel in a group or with their family. This contrasting potential for exposure influences the severity of this infection in children and hence the lesser intensity of exposure to Coronavirus is not surprising. The pathogenicity of MERS-CoV and SARS-CoV has been reported to be significantly less than the first-generation virus.[21–23] There are no reports in this regard for SARS-CoV-2.

Antigenic drift, a common occurrence in Influenza virus has also been reported for Corona namely the mutation D614G in the spike protein. Without change in pathogenicity, this antigenic drift for Coronavirus is implicated for higher viral transmission.[24] Whether this is a chance occurrence or causally related, is not clear. While the G614 variant of SARS-CoV-2 is the major strain spreading through Europe and the USA, the D614 variant of this virus has been spreading throughout China.[25] Whenever an infection is highly prevalent, the younger age groups get more affected. But this is not seen in COVID-19.

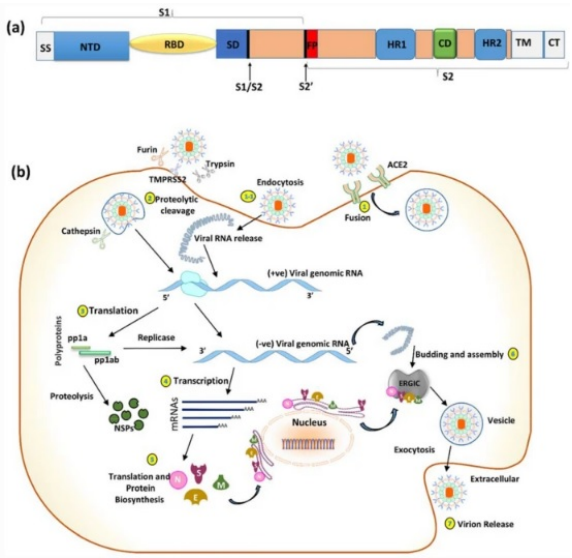

Fig. 2: An illustration of replication mechanism of coronavirus Ref. https://link.springer.com/article/10.1007/s15010-020-01516-2

1. Domain arrangement of SARS-CoV-2 spike protein. SS signal sequence, NTD N-terminal transactivation domain, RBD receptor binding domain, SD sub-domain, FP fusion peptides, HR1 heptad repeat 1, HR2 heptad repeat 2, CD connector domain, S1/S2 and S2′ protease cleavage sites, TM transmembrane domain, CT cytoplasmic tail.
2. Host cell entry and replication of SARS-CoV-2. SARS-CoV-2 infection starts with the binding of spike protein with ACE2 receptor and the invasion process is triggered by host cell proteases (furin, trypsin, TMPRSS2 and cathepsin). SARS-CoV-2 releases RNA into the host cell and the RNA are translated into viral replicase polyproteins (pp1a and pp1ab) and subsequently cleaves into NSPs. The full-length negative strand RNA copies of the viral genome are produced by the enzyme replicase using the full-length positive-strand RNA genome as a template. During transcription, RNA polymerase produces a series of sub-genomic mRNAs and translates into viral proteins [S (Spike), E (Envelope), N (Nucleocapsid), and M (Membrane)]. The viral proteins and the genome RNA are assembled into virions in Golgi and ER (endoplasmic reticulum), which are budding into ERGIC (ER–Golgi intermediate compartment) and released out of the cell via vesicles.

Less severe symptoms are being observed in kids and young adults with regard to the COVID-19 disease. But compared to illnesses brought on by the majority of the respiratory viruses which are more common and severe in children, this trend is completely different. Could this be explained by the variances that are observed in their oro-pharyngeal, lung, nasopharyngeal and gastrointestinal microbiota? This review addresses the potential of resident microbiota for the spectrum of expressions in susceptible population through various mechanisms. The nasopharynx of children is more extensively colonized with viruses and bacteria than adults, where microbial interactions and competition may limit the growth of SARS-CoV-2. One study found no discernible differences in the nasopharyngeal microbiota between SARS-CoV-2 patients and healthy individuals, whereas other investigations found significant differences in the oro-pharyngeal, lung and gut microbiota between these groups. In order to protect themselves from viral attacks, bacteria have devised their system in which they store chunks of assailing viral particles as repeating sets of genetic code within themselves.

Universal immunization program mainly focuses on the live attenuated vaccines in the first 2years of life when a child is born. This relationship between the off-target effect of childhood vaccines and the age-related variations seen in COVID-19 disease will be an area of research worth exploring. This has already engaged the attention of researchers. Randomized control trials (RCT) of BCG, MCV and OPV are already on. The results when available may provide a clue to the mechanisms of this off-target effect contributing to the age-related variations in the severity of COVID-19 infection.

2. Children and the Factors Protecting Them

2.1. Innate immunity and adaptive immunity

Innate immune response and trained immunity by epigenetic reprogramming contribute to the different responses to pathogens between children and adults.[26,27] NK cells which are the first line of defense are higher in number in children.

This first line of defense against SARS-CoV-2 is strengthened by the innate immune cells like NK cells through epigenetic reprogramming, which leads to memory.[27] This kind of a trained immunity reacts faster to any pathogen challenge.

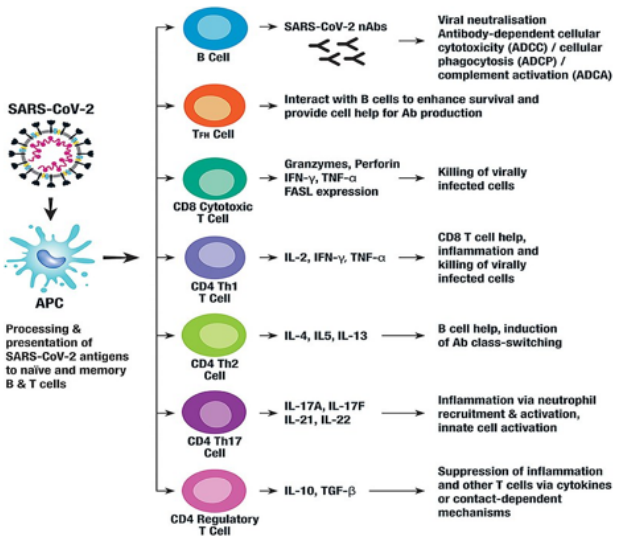

Fig. 3: Innate and adaptive immunity Ref.https://upload.wikimedia.org/wikipedia/commons/thumb/b/b3/Fimmu-11-579250-g003.jpg/800px-Fimmu-11-579250-g003.jpg?1618846054097

The mechanism of such a strong and enhanced protection does not explain the lack of protection for children from other respiratory viruses. Mucosal immunity and IFN production are important against SARS-CoV-2.[28,29] Challenging dendritic cells or epithelial cells of kids and adults to SARS-CoV-2 for comparing IFN production will be an interesting area of research.

The actual number of T cells and B cells with lymphocytes in greater proportion in children contrast with the age associated reduction in thymic function and naïve T-cells.[30,31] This strong naïve T-cells and the immune response mediated by T-cells in children against decreased lymphocytes in SARS-CoV-2 infected adults play an important role in protecting children.[32] A muted or poorly mounted pro-inflammatory cytokine storm in children could

cause the differences in severity of the Coronavirus infection in them despite similar quantum of exposure to infection as in adults.[30,33,34] Children hospitalized with COVID-19 infection had increased serum levels of IFN and IL-17A but the serum levels of IL-6 and TNF-α remained the same.[16] Such an age-related variation in cytokine storm (ARDS) due to Influenza infection or RSV however has not been reported.[34,35]

2.1.1. Frequent recurrent and concurrent infections

Commonly circulating human Corona viruses (HCoVs)[36,37] could inhibit the replication process of SARS-CoV-2.[38] Easy clearance of SARS-CoV-2 is facilitated by modifications in trained immunity and improved innate immune system activation.[39]

2.2. Cross-reactive Coronavirus antibodies

Various studies suggest that children and young adults may be protected from SARS-CoV-2 infection because they already have cross-reactive antibodies from more recent and common Human Coronavirus (HCoV) infections.[40] Children with SARS-CoV-2 infection and those who were not infected had similar antibody levels against HCoVs.[41] The virus would not bind with the SARS-CoV-2 receptor binding domain (RBD) since they are non-neutralizing antibodies.[42–45] Adults, particularly elderly people, have higher levels of neutralizing and non-neutralizing antibodies than young adults and children.[16,41,46] IgA antibodies present on the mucosal surfaces are important as a defense mechanism against SARS-CoV-2.[29]

2.2.1. Colonizing Microbiota

The variations in the microbiota (oropharyngeal, nasopharyngeal and gastrointestinal) may account for less severe manifestations in children. Regulation of immunity, inflammation and containment of pathogens are important functions of the microbiota.

ACE-2 being highly expressed in gastrointestinal tract and respiratory tract, SARS-CoV-2 might therefore affect inflammation through the microbiota.[47,48] Age-related variations in the gut microbiota with higher numbers of *Bifidobacterium* in Children have been observed.[21,49] A lesser bacterial load and diversity in *Faecalibacterium* and a higher relative abundance of *Bacteroides* and *Faecalibacterium* have been reported in the gut microbiota of individuals infected with SARS-CoV-2.[50–52] Decreased gastrointestinal ACE-2 expression of *Bacteroides* and anti-inflammatory properties of *Faecalibacterium* have been implicated. Whether prior antibiotic administration and the diet in a particular culture can influence the resident microbiota for its interaction with SARS-CoV-2 are areas for further study.[53]

2.2.2. Off-target effects of vaccines

Epidemiologists and Vaccine scientists have been focusing only on the specific effects of vaccine against the pathogen (eg: BCG for Tuberculosis, MMR for Measles, Mumps and Rubella) so far. However, the immunomodulatory effects of vaccines beyond protection against the target disease are receiving greater attention on the non-specific – off target effect.[53–58] Investigations on the immunomodulatory effects of vaccines and their underlying mechanisms are ongoing.

The BCG vaccine has an influence on the T-cell immunity and natural immunity through epigenetic reprogramming of immune cells and also by the altered cytokine responses.[59–61] This was evident in the reduction of all-cause mortality and protection against viral infections by BCG and MCV.[62–64]

An increasing awareness of Universal vaccination in the current times was cited as a reason for this adult and child differences.[65–67] However, studies on a nation's BCG vaccination guidelines and the intensity of the COVID-19 outbreak showed no difference in the COVID-19 infection rate in BCG-vaccinated people compared to BCG-naïve people decades after vaccination.[68] Randomized control trials of BCG are ongoing.[69–74] The immunomodulatory effects of another live attenuated vaccine (MCV) showed a reduction in circulating lymphocytes, CD4 cells but an increase in CD8 cells.[75,76] Thus, studies to assess the effect of BCG and MMR in mitigating COVID-19 disease are underway.[77–79]

3. Factors Affecting Adults

3.1. The ACE2 receptor and TMPRSS2

Angiotensin Converting enzyme 2 (ACE2) is a receptor that is present on the epithelial cells of the lungs, oropharynx, nasopharynx, kidneys, heart and few other organs.[80] This receptor tends to be the major reason for the entry of the virus SARS-CoV-2 into the host. It has been found that children's ACE2 expression has a very low affinity for SARS-CoV-2 when compared with adults. But this affinity of ACE2 receptors for the virus increases as we age and few other factors like the dietary habits, smoking and genetics can also influence this phenomenon.[81–83] Few patients who take ACE inhibitors for arterial hypertension have been found to express a greater number of ACE2 receptors than usual and this makes them more prone and susceptible to the infection. But it is still unclear if there is an interrelation between the severity of the COVID-19 infection and the levels of ACE2 receptors in the host epithelial cells.[84]

Transmembrane protease serine 2 is an enzyme which is encoded by the TMPRSS2 gene in humans. The entry of the SARS-CoV-2 into the cells of the host is also facilitated by the Transmembrane serine protease 2 (TMPRSS2) that activates the spike protein domain on the virus. This helps the virus in fusing to the respiratory epithelial cells through

Table 1: Factors that protect children and its mechanism

S.No	Factors protecting children	Hypothesis	Proposed mechanism
1	Immune system	Age-related differences in immune response	Stronger innate, trained immune response leading to more effective virus containment/ clearance
			Weaker adaptive immune response and therefore less hyperinflammation
			Lower proinflammatory cytokine responses (Cytokine storm)
2	Recurrent and concurrent infections	Viral and mycoplasma infections	More frequent infections with other pathogens may help fight SARS-CoV-2
3	Cross-reactive Coronavirus antibodies	Exposure to commonly circulating HCoV (229E, HKU1, NL63, OC43)	Pre-existing neutralizing antibodies and T-cell immunity to commonly circulating HCoV in younger age groups cross protect against SARS-CoV-2
4	Microbiota (Nasopharyngeal, oropharyngeal, lung and gastrointestinal)	Colonizing microflora	Differences in the microbiota might influence susceptibility to SARS-CoV-2
5	Off-target effects of live vaccines	Trained immunity from BCG, MCV, OPV	More recent vaccination with live vaccines that have off-target effect
6	Exposure	Intensity of viral exposure	Severity of COVID-19 associated with initial viral load

*Adapted from Zimmermann P & Curtis N, 2020[77]

Table 2: Factors increasing the risk in adults and its mechanism

S.No	Factors increasing the risk in adults	Hypothesis	Proposed mechanism
1	Pre-existing immunity	Cumulative exposure to commonly circulating HCoVs (229E, HKU1, NL63, OC43)	Non-neutralizing HCoV antibodies facilitating cell entry and viral replication (Antibody dependent enhancement)
2	ACE2 receptors and TMPRSS2	Viral entry	Age-related differences in expression, affinity and distribution facilitate SARS-CoV-2 entry into cells.

*Adapted from Zimmermann P & Curtis N, 2020[77]

the binding to ACE2.[85]

3.1.1. Low levels of Vitamin D

Vitamin D is rich in anti-inflammatory properties and vitamin D deficiency can increase the risk for developing respiratory infections in humans. Vitamin D protects us from the viral antigen through various mechanisms like reduced synthesis of inflammatory cytokines, killing of the viral antigen and prevention from the infiltrates entering into our lungs.[86] It has been found that supplementation of vitamin D for adults could be a prophylactic measure for COVID-19 disease.[87]

3.1.2. Comorbidities

Comorbidities observed in patients with COVID-19 disease include obesity, hypertension, heart disease and diabetes.[88] These patients are at a higher risk when compared with others. The dietary habits, smoking or alcohol consumption by a person could affect their health and also the genetic makeup could be a major deciding factor which could determine these comorbidities in a person. Children with asthma or other respiratory problems and cardiovascular problems need to be taken care of and treated properly.[89]

3.2. Future prospects

The severity of the COVID-19 disease observed in patients based on the age-related differences must be further explored. A lot of researches are ongoing in this aspect. If a mutated variant of this virus emerges in future, the symptoms and severity of the disease could vary and depend upon the mechanism of action of the virus in the host.

4. Conclusion

The spread of SARS-CoV-2 and the role of children and adults associated to it are still uncertain. Children, in particular, have lower susceptibility to the COVID-19 infection when compared to adults and are also less likely to

develop a chronic disease even if infected and thus the rate of transmission by children is very less. This difference is seen between children and adults mainly because of various factors such as the off-target effect of childhood vaccines and intensity of viral exposure. Few of the other proposed explanatory mechanisms for this lower propensity to disease among children may include the age-dependent expression of Angiotensin converting enzyme (ACE-2) gene and also the differences seen in innate and adaptive immunity. Frequent exposure to various respiratory infections and also an individual's pre-existing immunity to the Coronaviruses may decide upon the severity of the infection.

The SARS-CoV-2 pandemic is definitely going to have a long-lasting impact in our World. There are modified clinical trials which are on-going. New research paradigms will emerge because of this pandemic. Understanding the mechanisms underlying the age-related variations in COVID-19 severity would provide newer insights for the management and cure of this novel infection.[90]

5. Why this Topic for Review?

Respiratory viral infections are a common occurrence in children worldwide.

It need not be over-emphasized that respiratory viral infections are always higher in children (RSV, Influenza and Parainfluenza virus) but in the unprecedented first wave of the pandemic, the COVID-19 infection was reported slightly less typical and less severe among children compared to adults. All of us are learning to live with this novel infection with rays of hope through vaccination strategies. This continuing battle against COVID-19 is going to give a better understanding of the larger meaning of little things for a context-dependent COVID-19 control. The period of study of this research work is from December 2019 to December 2020.

6. Scope of this Review

1. COVID-19 virus- Immune system and cross-reactive microbiota.
2. Off-target effect of childhood vaccines-BCG and MCV.
3. Vaccine platforms- New Research paradigms in all aspects are emerging because of this pandemic.

7. Abbreviation

1. ACE-2: Angiotensin Converting Enzyme-2
2. ARDS: Acute Respiratory Distress Syndrome
3. BCG: Bacillus Calmette-Guerin
4. CD-4 and CD-8: Cluster of differentiation
5. COVID: Coronavirus Disease
6. HCoV: Human Coronavirus
7. IFN: Interferon
8. IL: Interleukin
9. MCV: Measles containing vaccine
10. MERS: Middle-East Respiratory Syndrome
11. MMR: Measles, Mumps and Rubella
12. NK cells: Natural Killer cells
13. OPV: Oral Polio Vaccine
14. RBD: Receptor Binding Domain
15. RCT: Randomized Control Trials
16. RSV: Respiratory Syncytial Virus
17. RT-PCR: Reverse Transcription- Polymerase Chain Reaction
18. SARS: Severe Acute Respiratory Syndrome
19. TMPRSS-2: Transmembrane protease, Serine-2
20. TNF: Tumor Necrosis Factor

8. Source of Funding

None.

9. Conflict of Interest

The authors declare no conflict of interest with regards to the publication of this research review article.

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