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ZINC SUPPLEMENTATION ALTERED BRONCHUS MUCOSAL IMMUNE STATUS EXPRESSED BY IFN- γ , IL-6, DENDRITIC CELLS AND sIgA

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ABSTRACT

Zinc deficiency can cause suppression of the immune system and make it susceptible to infection, including infection of the respiratory tract. The benefits of zinc have not been widely studied and known to improve the bronchial mucosal immune status during respiratory tract infections. The aim of this study was to analyze the alteration of the bronchus mucosal immune status, expressed by IFN- γ , IL-6, dendritic cells and sIgA, that was caused by zinc deficiency. Twenty-four *Rattus norvegicus* strain Wistar species were divided into 4 experimental groups consisting of normal zinc + zinc supplementation (Z1), normal zinc (Z2), zinc deficiency + zinc supplementation (Z3) and zinc deficiency (Z4). The dose of zinc supplementation was 60 ppm in the normal diet group and 120 ppm in the zinc deficiency group. Dendritic cells, sIgA and the cytokines IFN- γ and IL-6 were examined using immunohistochemistry to assess bronchial immune status. The results of this study showed that dendritic cells and sIgA increased ($p < 0.0001$) in the Z3 group but that cells producing cytokine IL-6 and IFN- γ ($p < 0.0001$) decreased in the Z3 group. In the Z1 group, a statistically significant ($p < 0.0001$) increase was seen in the number of dendritic cells and sIgA as well as an increase in cells producing cytokine IL-6 ($p < 0.016$) compared with the Z2 group. According to the results, zinc is proven to alter the immune mucosa of the bronchi. Zinc supplementation can reverse the immune status by changing the dendritic cells, sIgA and cytokines IFN- γ and IL-6 in a zinc deficiency condition.

1. Introduction

In developing countries, respiratory tract infections are a major cause of morbidity and mortality in children under 5 years old (Rudan, 2004). In Indonesia, respiratory infections cause about 15.5% of mortalities in children aged 1-4 years old (Risksedas, 2013). This is thought to be due to the immunological decline that occurs in malnourished children (De Francisco, 1993).

There is no complete study of the use of zinc with respiratory infections, but there is a relationship between zinc deficiency and respiratory diseases (Krebs, 2000). Zinc stimulates the immune response by activating

dendritic cells and releasing cytokines to coordinate the immune response so that there is a balance of Th1 and Th2 (Haase, 2009).

Zinc is an essential substance in the cellular and humoral immune system components (Zalewski, 1996). Until now, there has been no research done on the effect of zinc that has been given orally on the immune status and immune response of dendritic cells, sIgA, IFN- γ and IL-6 in the bronchial mucosa of rats with normal zinc levels and rats with a zinc deficiency.

This study was conducted to prove that zinc alters the immune mucosa of the bronchi. This

mechanism is explained more thoroughly in experiments that were designed to determine the relationship between immune activation of dendritic cells, sIgA, IFN- γ and IL-6 in individuals with both normal zinc levels and zinc deficiency. This experiment used rats as subjects since the treatment and final inspection procedures have been proven to be fatal in human subjects.

2. Materials and methods

2.1. Animals

This research was ethically approved by a committee in the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. Twenty-four white rats, *Rattus norvegicus* strain Wistar species, that fulfilled the inclusion criteria (healthy males, aged 5-6 weeks, weight ranging from 50 to 100 grams) were divided into 4 groups: normal zinc + zinc supplementation (Z1), normal zinc (Z2), zinc deficiency + zinc supplementation (Z3) and zinc deficiency (Z4). The dose of zinc supplementation was 60 ppm in the normal diet group and 120 ppm in the zinc deficiency group. The treatment took 42 days, and on day 42 a necropsy was performed.

2.2. Zinc Administration

The number of meals per day was determined by 10% of the body weight of each rat. The pellets without the zinc content included 25% protein, 5% fat and 50% carbohydrates. Drinking water was supplied ad libitum with the drop method using distilled water to avoid contamination with dirt. Zinc deficiency conditions were made based on the method used in a study by Soemyarso et al. (2019). Zinc was administered via an oral tube in 30 ppm (30 mg zinc/kg feed/day) with 0.2% zinc sulfate syrup in Groups Z1 and Z2, in 0.5 ppm (0.5 mg zinc/kg feed/day) with 0.002% zinc sulfate syrup in Groups Z3 and Z4, in 60 ppm (60 mg zinc/kg feed/day) with 0.4% zinc sulfate syrup in Group Z1 and in 120 ppm (120 mg zinc/kg feed/day) with 0.8% zinc sulfate syrup in Group Z3.

2.3. Bronchial Tissue Collection

On day 42, a necropsy was performed. The bronchial organs were cleaned and fixed in 10% buffered formalin solution, followed by dehydration, clearing, impregnation and embedding. Then, experts from the Pathology Anatomy Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, fixed the organs by using the paraffin method. Expression of the cytokine-producing cells was detected with monoclonal antibodies (dendritic cells [Follicular DC Marker Antibody (Ki-M9R) sc-58529], IL-6 [IL-6 antibody (E-4)], sIgA [Sigma Receptor Antibody (B-5) sc-137075] and IFN- γ [IFN gamma Antibody Bioss Inc.]) before it was seen through a Nikon E100 microscope (Nikon Instruments Inc. [magnification 400x]) in the Biochemistry Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang. The number of dendritic cells, sIgA and cytokines IFN- γ and IL-6 was determined by counting the number of cells per incision.

2.4. Statistical Analysis

Descriptive analysis was used to determine the results of the observations and profiles of the cells producing dendritic cells, sIgA and cytokines IFN- γ and IL-6 for each group. Expression data on the number of cells producing dendritic cells, sIgA and cytokines IFN- γ and IL-6 are expressed in mean \pm standard deviation. Multivariate analysis of variance was used to analyze the effect of zinc on the changes in the number of cells producing dendritic cells, sIgA and cytokines IFN- γ and IL-6 in the bronchial mucosa in all treatment groups. To find the comparison between the treatment groups, a double comparison LSD test was performed. Data were analyzed using a 95% confidence level ($\alpha = 0.05$).

3. Results and discussions

This study used an immunohistochemical staining method. Then, a microscope was used to observe the dendritic cells, sIgA and cytokines IFN- γ and IL-6 on the bronchial tissue (Figure 1).

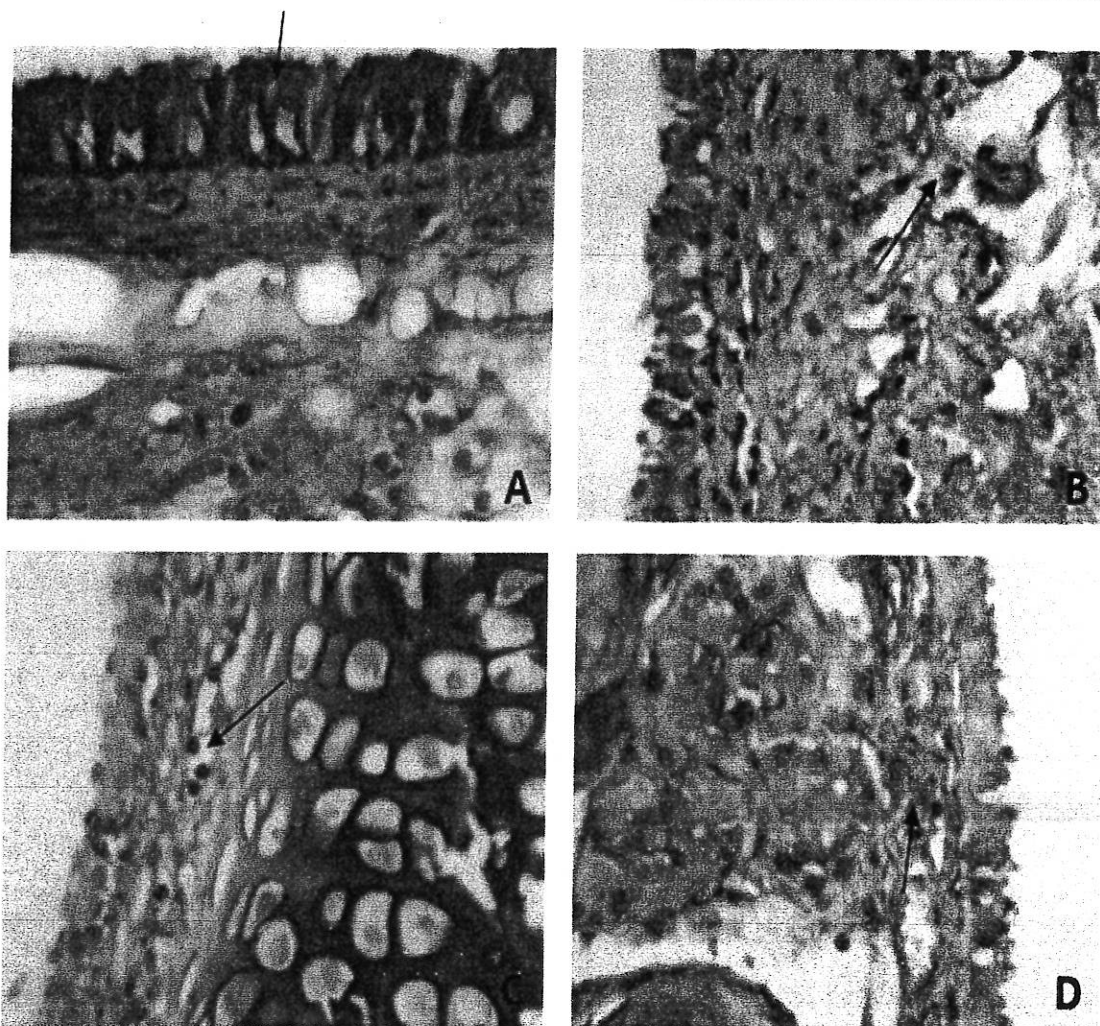


Figure 1. Rat's bronchus with immunohistochemical staining with monoclonal anti-mouse A) sIgA; B) IFN- γ ; C) dendritic cell; D) IL-6 with 1000x magnification.

The effect of zinc supplementation on the immune status of the mucosal bronchus reflected by sIgA-producing cells, dendritic cells and cytokines IFN- γ and IL-6 in the zinc-deficient rats can be seen below (Figure 2).

When zinc-deficient rats are supplemented with zinc, changes in the immune status can be seen in the form of a statistically significant increase ($p < 0.0001$) in bronchial mucosal cells producing secretory IgA and dendritic cells

compared with the rats with a zinc deficiency. However, a statistically significant decrease ($p < 0.0001$) is seen in cells producing cytokine IL-6 and IFN- γ compared with the rats with a zinc deficiency, so zinc can be said to be beneficial for the zinc-deficient rat in sIgA immune response and dendritic cells but not in the immune response of IL-6 and IFN- γ (Figure 2).

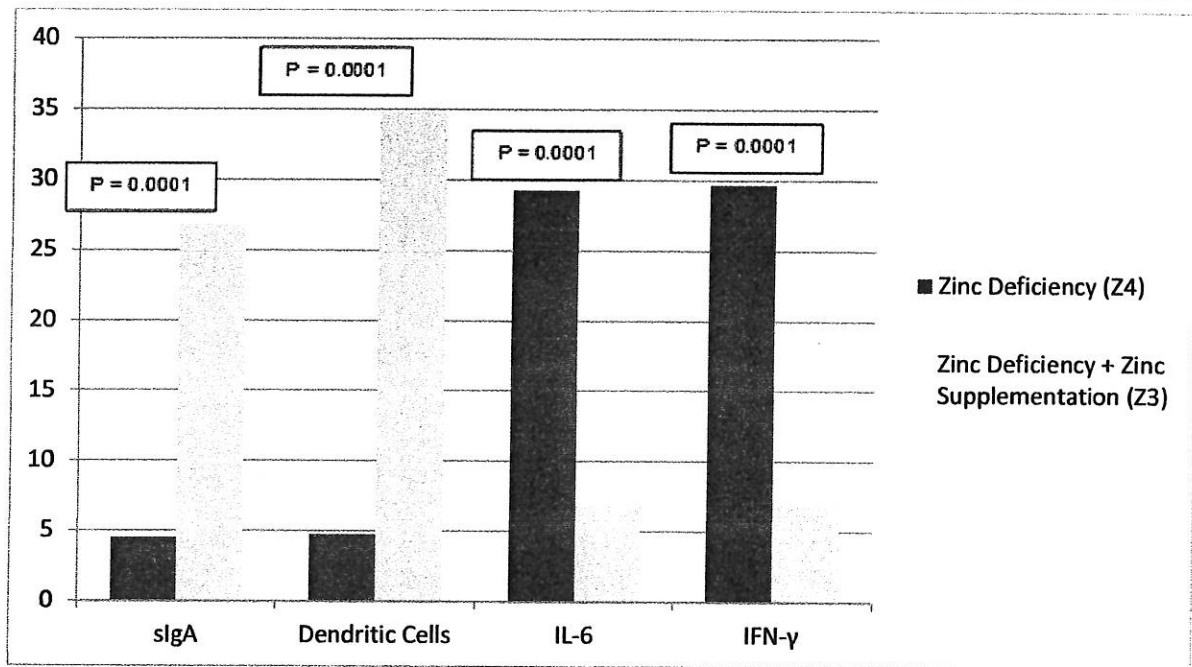


Figure 2. A profile comparing the cells producing sIgA, dendritic cells and the cytokines IFN- γ and IL-6 of the zinc-deficient group with the zinc deficiency + zinc supplementation group.

The effect of zinc supplementation on the immune status of the mucosal bronchus reflected by cells producing sIgA, dendritic cells and cytokines IFN- γ and IL-6 on a rat with normal zinc levels can be seen below (Figure 3). When rats with normal zinc levels are supplemented with zinc, changes in the immune status can be seen in the form of a statistically significant increase ($p < 0.0001$) in bronchial mucosa cells producing secretory IgA and dendritic cells compared with the rats with normal zinc levels. There is also a statistically significant increase ($p < 0.016$) in cells producing cytokine IL-6 compared with the rats with normal levels of zinc. The increase also occurs in cytokine IFN- γ but is not statistically significant ($p < 1.000$) compared with rats with normal zinc levels, so zinc is determined to be beneficial in improving the immune status in rats with normal levels of zinc (Figure 3).

This study was conducted to prove that zinc deficiency alters the immune mucosa of the bronchi and to seek evidence regarding the effect of zinc administration on the bronchial immune system expressed by changes in dendritic cells, sIgA and cytokines IFN- γ and

IL-6 of zinc-deficient rats and rats with normal zinc levels.

In the study conducted by Prasad, zinc deficiency also resulted in stress and activation of monocytes and macrophages, resulting in an increased generation of the inflammatory cytokine IL-6. In observational studies, individuals with normal post-intervention or serum zinc concentrations have a shorter bout of pneumonia. This is also evident in studies conducted by several student officers that show zinc may prevent upper respiratory tract infections (Prasad, 2009). In the same year, Prasad also reviewed the effects of zinc in patients that were infected with TB. It was found that zinc stimulated an increase in IFN- γ .

In 2004, Wieringa et al. conducted a study on 59 infants aged 3-10 months. They measured and compared levels of IFN- γ and IL-6 in infants with both normal zinc levels and zinc deficiency levels and on stimulation with LPS (Lipopolysaccharide). Results were obtained that showed a decline in the levels of the cytokines IFN- γ and IL-6 after stimulation with LPS in both normal zinc and zinc deficiency conditions.

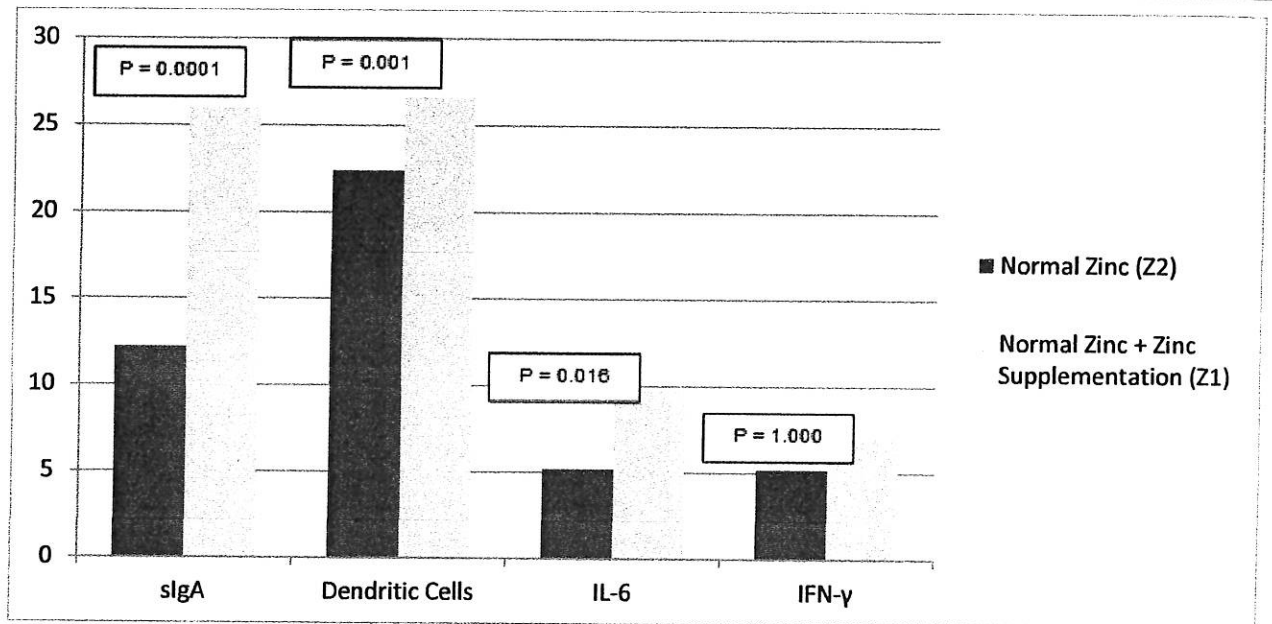


Figure 3. A profile comparing the cells producing sIgA, dendritic cells and the cytokines IFN- γ and IL-6 of the normal zinc group with the normal zinc + zinc supplementation group.

In a study, Brandtzaeg (2007) suggested that the effector site is where sIgA is secreted through the mucosal epithelium. Antigen presentation and activation of B cells in one area of the mucosa can result in sIgA appearing in the mucosa of different organs in accordance with the principle of the “common mucosal immune system” (Brandtzaeg, 2007).

In the same study, he also explained that the inductive sites for mucosal immunity are initiated by regional MALT, in which exogenous antigens will be streamed actively to achieve the antigen presenting cell (APC), including the dendritic cell (DC) and macrophage and B cells. An intra- or subepithelial dendritic cell that is not moving will capture antigens on effector sites and will migrate through the lymphatic flow toward the local lymph nodes and then become an active APC that stimulates T cells that will be productive or suppress the immune response. Once recognized as B cells and effector T cells, they will be streamed from MALT and the lymph nodes into the bloodstream to extravasation at mucosal effector sites (Brandtzaeg, 2007).

Holmgren and Czerkinsky (2005) proved the presence of kinetic-producing cells with specific IgA antibodies in peripheral blood circulation

after administration of oral immunization in humans. They also showed that, after being given oral bacterial antigens, the cells that will secrete specific IgA antibodies appear in the peripheral blood circulation and are followed by an increase of the sIgA antibodies in saliva and tears. These studies also support/justify the concept of the common mucosal immune system in humans, and they also showed that oral immunization can be effectively used to induce the common sIgA antibodies in several external secretions (Holmgren and Czerkinsky, 2005).

Some evidence has suggested that mucosal surfaces, including the gastrointestinal and respiratory tract, are part of the common mucosal immune system and that both B and T lymphoblasts from one area of the mucosa in particular will return to the mucosal areas that are as far away as the original area. In research conducted on respiratory tract secretions in sheep, IgA is the major Ig in the upper respiratory tract (nasopharynx, trachea and main bronchi), and the large of numbers of IgA plasma cells reflected on the respiratory mucosa suggest that some of these cells may be derived from distant mucosa areas or elsewhere (Scicchitano, 1984).

Studies concerning the effects of zinc supplementation in pregnant rats conducted by

Raqib et al. (2007) found that administration of zinc actually decreases IgA levels. This finding contrasts with the results of our study where bronchial sIgA levels increased in rats with normal zinc levels that were given zinc supplementation.

New Research Findings

This study has provided new findings with evidence that zinc administration can improve the immune system in rats with normal zinc levels and rats with a zinc deficiency. The immune response in bronchial mucosa increases dendritic cell-producing cells, sIgA, IFN- γ and IL-6. Several studies on the effect of zinc were limited to the clinical improvement of respiratory tract infections, but no studies have been done in terms of changes and improvements in the immune system.

4. Conclusions

Zinc is proven to alter the immune mucosa of the bronchi. Zinc supplementation can reverse the immune status by changing dendritic cells, sIgA and the cytokines IFN- γ and IL-6. Further research is needed both immunologically and clinically on the use of zinc as a supplementation for the prevention of possible exposure to oral pathogens that may affect the bronchus.

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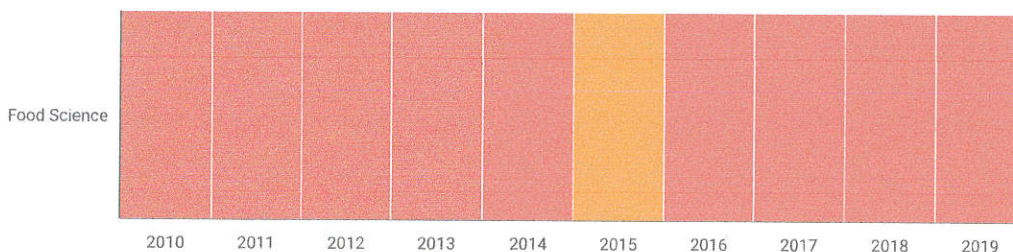
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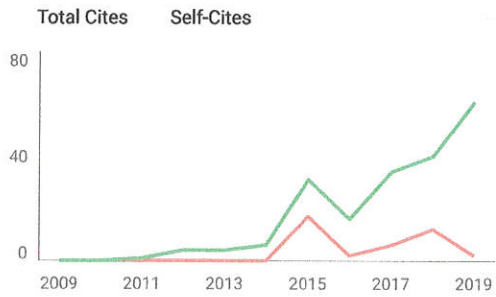
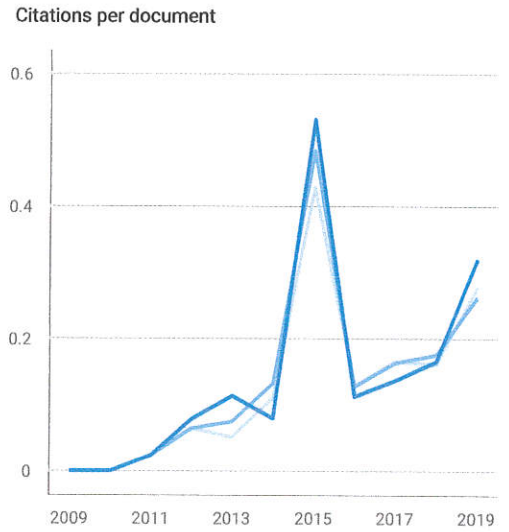
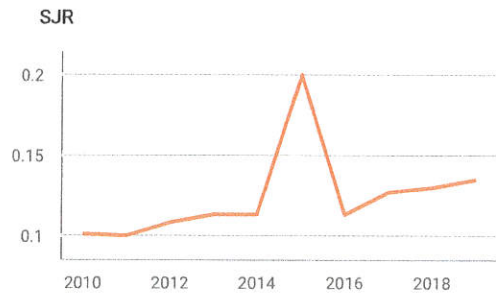
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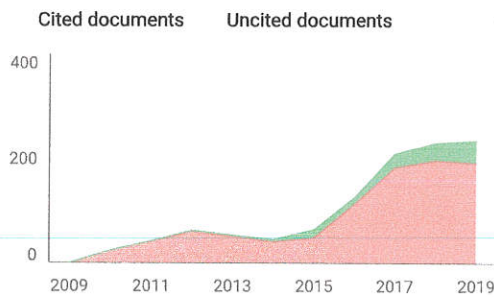
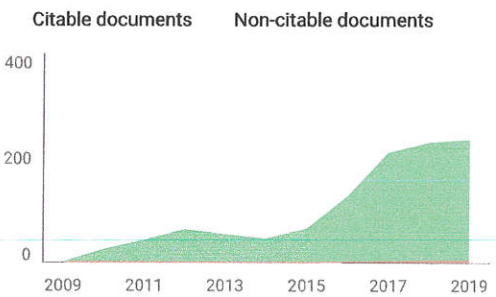
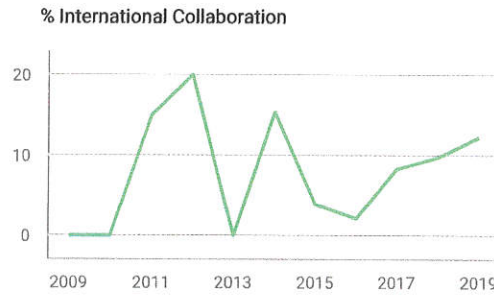
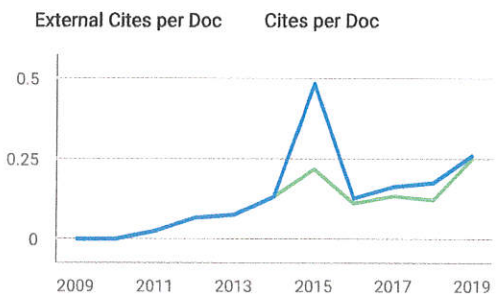
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