To study the correlation of serum ST-2 levels with absolute eosinophil count in COPD patients

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KEYWORDS

COPD, ST-2 , IL-33, AEC, Smokers, Non-smokers

ABSTRACT

Background: COPD is chronic lung disease associated with frequent disease exacerbations and poor quality of life. COPD patient with eosinophilic inflammation phenotypes are under increased risk for exacerbation. Thus the master switch of inflammation i.e.IL-33/ST-2 pathway can be blocked by using anti-ST2 monoclonal antibodies and further complication of COPD can be minimized. Thus we aimed to study the correlation of absolute eosinophil count with ST2 in COPD patients. In order to investigate the concentration of serum ST-2 (suppression of tumerogenicity 2) receptor and its correlation with absolute eosinophil count we recruited 150 COPD patients and 150 healthy controls in our hospital and divided into two subgroups; smokers and non-smokers. When we compared serum ST-2 of COPD patients with healthy controls, a significantly higher level of serum ST-2 was found in COPD patient as compared to healthy control. The positive correlation of AEC and ST2 in COPD patients as well as healthy control subjects were recorded, which confirms the role of ST2 receptor mediated eosinophilic inflammation in COPD patients via IL-33 activation.

Introduction:

Chronic inflammation is a major hallmark of COPD which accounts for major economic burden globally[1,2]. The unresolved inflammation is due to tobacco smoking, allergens, occupational exposure and indoor or outdoor pollutants exposure[2]. Chronic insult with these all stimuli causes lung tissue injury and damages the cells, which alert the immune system about the danger and activates the immune system as a response to this inflammatory signal. This results in the release of IL-33 as an alarming signal[3,4]. IL-33 acts as a barrier between trigger factors and activation of innate and adaptive immune system cells after binding with its ST-2 receptor, which triggers downstream pathway through the secretion of other cytokines such as IL-13, IL-4 IL-5 etc. This results in the increased expression of neutrophils, eosinophils, macrophages and dendritic cells, mast cells, type 2 helper T-cells and ILC2(type 2 innate lymphoid cells)[4,5] . Eosinophilic inflammation is also up-regulated by ILC2 cells activation along with IL-13 , IL-5 production in

response to IL-33 release[5,6,7]. IL-4, Il-5 ,IL-13 are required for eosinophil survival and maturation[4,6]. The ST-2 is heterodimeric in nature which exist in both secretory and transmembrane form. The transmembrane form consist an intracellular Toll/IL-1 receptor (TIR) domain, extracellular IgG-like domains and a transmembrane domain. Il-33 binds with extracellular domain of ST-2 which activates MyD88 dependent abnormal inflammatory pathway after dimerization of TIR domain[8,9]. The inflammatory microenvironment induces eosinophilic inflammation with increased eosinophil cell count [2]. Thus IL-33/ST-2 pathway along with increased eosinophil is a key regulator for COPD progression and exacerbation [4]. The anti-inflammatory targeted corticosteroids are most effective treatment for eosinophilic inflammation in COPD but this therapy increases risk of infection in patients. Thus herein this article we tried to describe current and future approaches by targeting master switch i.e. IL-33/ST-2 pathway blockage to decrease eosinophilic inflammation and to reduce rates of severe exacerbation in COPD patients, because increased eosinophilic inflammation is associated with higher risk of severe exacerbation which is the most common cause of death in COPD[4,6]. In previous studies, eosinophilic inflammation and exacerbation was normalized by using IL-33 blockage via reducing the ST-2 infiltration in a mouse model of persistent and exacerbating airway disease[4]. Currently some clinical trial using anti-Il-33/ST-2 or anti-ST-2 monoclonal antibodies at phase 1, 2 & 3 are under evaluation for COPD treatment [10] . Thus we aimed to study correlation of absolute eosinophil count with ST-2 levels in COPD patients to find out that COPD subgroup with higher eosinophilic count will be more effective in anti-IL-33/ST-2 therapies.

Material and method: - The aim of present study is to study the correlation of AEC with ST-2 in COPD patient by estimation of serum concentration of ST-2 and AEC to find out that COPD subgroup with higher eosinophilic count will be more effective in anti-IL-33/ST-2 therapies. The objectives of our study are to assess the serum concentration of ST-2 and absolute eosinophil count in COPD patients and healthy control subjects with cigarette smoking and without cigarette smoking and to study the correlation of absolute eosinophil count with serum ST-2 in healthy subjects and COPD patients. Present prospective study was done at Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun. It includes 150 COPD patients and 150 healthy control from Shri Mahant Indresh hospital, Dehradun who were more than 40 years of age at the time of enrolment into the study. COPD was diagnosed in accordance with GOLD guidelines, which was made on spirometry with post bronchodilator $FEV_1/FVC < 0.70$ in Respiratory department of Shri Mahant Indresh hospital[11]. Participants of smoker group (both COPD & healthy control) must had a history of more than 10 pack years of smoking. COPD patients was on their regular treatment for COPD. Each patient had a documented history of $\geq$ 2 moderate or $\geq$ 1 severe COPD exacerbation within 12 month prior to enrolment. The informed consent was taken from all participants. Blood sample was collected form each participants after overnight fasting by venipuncture from medial cuboidal vein in a plain vial for ST2 and EDTA vial for AEC estimation. The supernatant serum was separated with centrifugation technique at 3000rpm. Then the separated serum was used for ST-2 estimation based on ELISA technique. The normal range for ST-2 was between 0.31- 20ng/ml [12]. Here sST-2 (soluble ST-2) form of ST-2 was measured. The AEC was estimated by using Beckman coulter counter [13]. The mean and standard deviation value of ST-2 and AEC in both COPD (smoker and non-smoker) and healthy subjects (smoker& non-smokers) were calculated by using Microsoft excel. The difference of ST-2 and AEC between COPD and healthy groups were compared by student's t-test and Pearson's correlation coefficient via using SPSS statistical software. The difference of ST-2 and AEC between healthy smokers versus non-smokers, COPD smokers versus non-smokers were also analyzed using the student's

t-test and Pearson's correlation coefficient to find out significant difference. We used microsoft excel to find out correlation coefficient between AEC and ST-2 by plotted a scattered diagram according to the distribution of variables.

Result and Discussion:

Table-1: Comparison of Study group (COPD) with healthy control

Parameter	Group	Mean± SD	p-Value
Absolute eosinophil count (/ cumm)	COPD	225.73±146.65	<0.0001***
	Control	123±57.48	
ST-2 (ng/ml)	COPD	10.72±7.44	<0.0001***
	Control	3.57±2.28	

P<0.01=significant*, P<0.001=very significant, P<0.0001=extremely significant*****

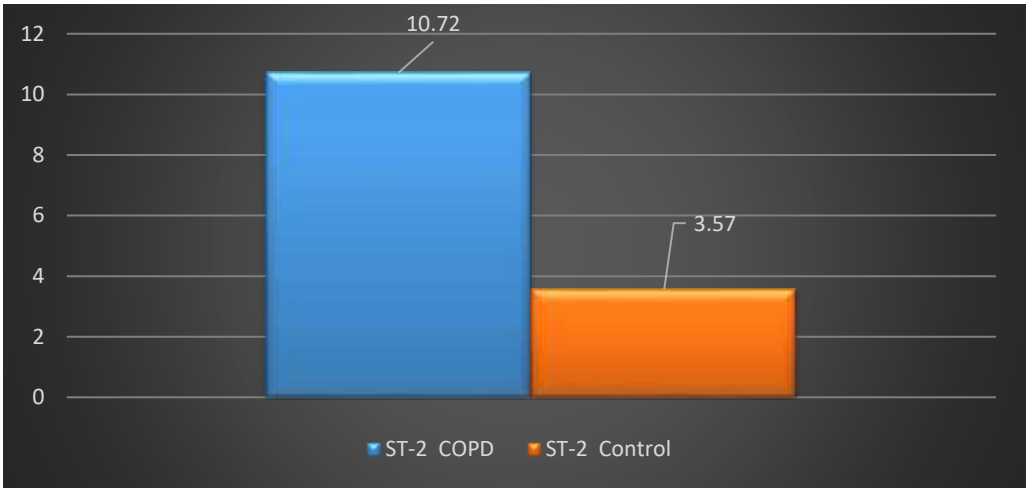

Figure 1: Comparison of ST-2 (ng/ml) in COPD and control group

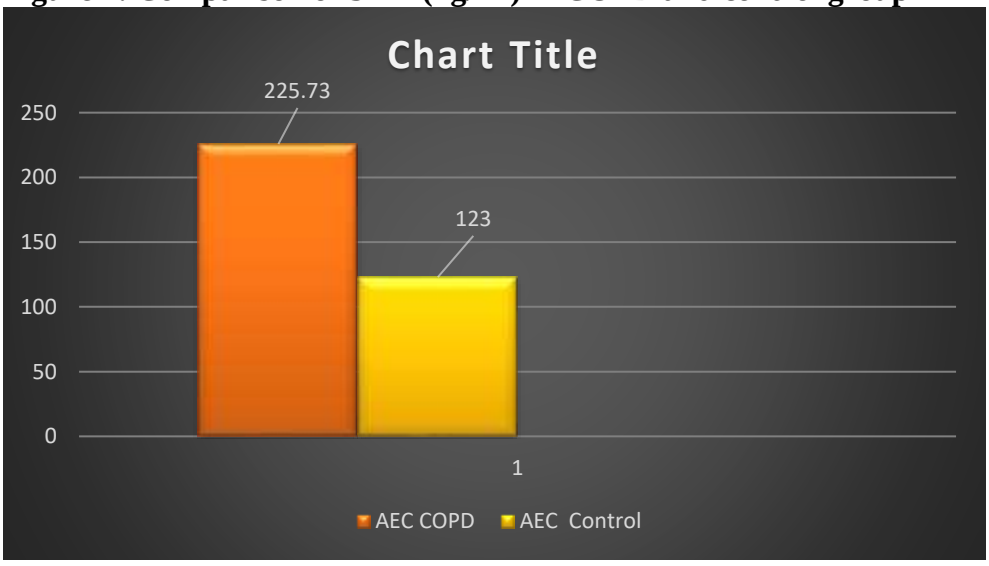

Figure 2: Comparison of AEC (/cumm) in COPD and control group

Table-2: Comparison of COPD (Smoker & Non-Smoker) and healthy control (Smoker & Non-Smoker)

Parameter	Group		Mean± SD	p-Value
AEC(/cumm)	COPD	Smoker	239.12±156.22	0.0588
		Non-Smoker	187.59±108.01	
	Control	Smoker	142.12±54.07	<0.0001
		Non-Smoker	68.39±19.05	
ST-2(ng/ml)	COPD	Smoker	11.55±7.88	0.021
		Non-Smoker	8.36±5.46	
	Control	Smoker	4.36±7.49	<0.0001
		Non-Smoker	1.31±0.81	

P<0.01=significant*, P<0.001=very significant, P<0.0001=extremely significant*****

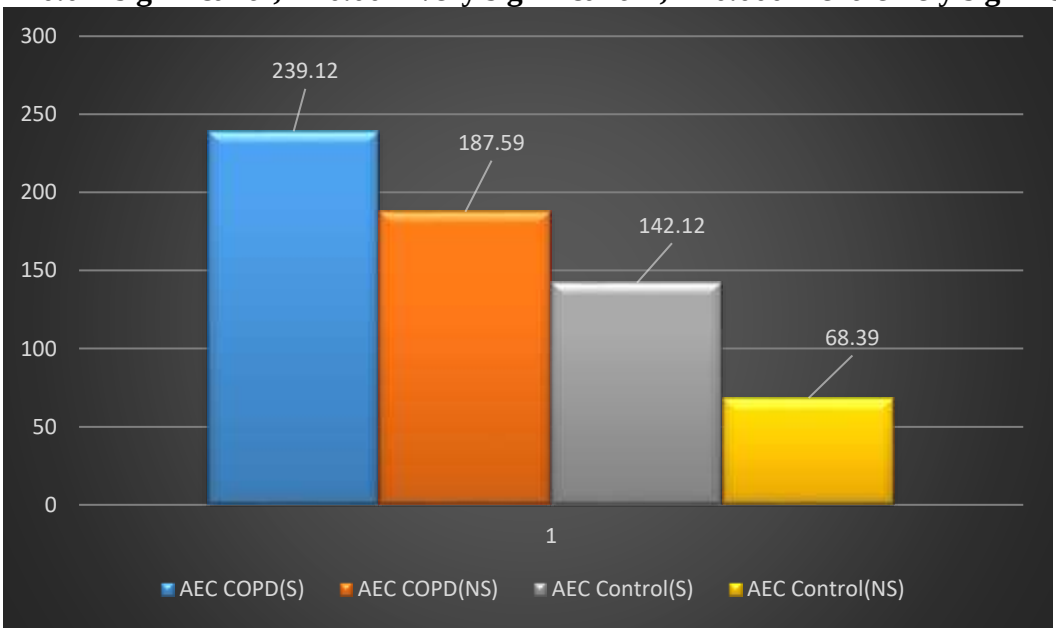

Figure 3: Comparison of AEC(/cumm) in Smoker and non-smoker COPD and control groups

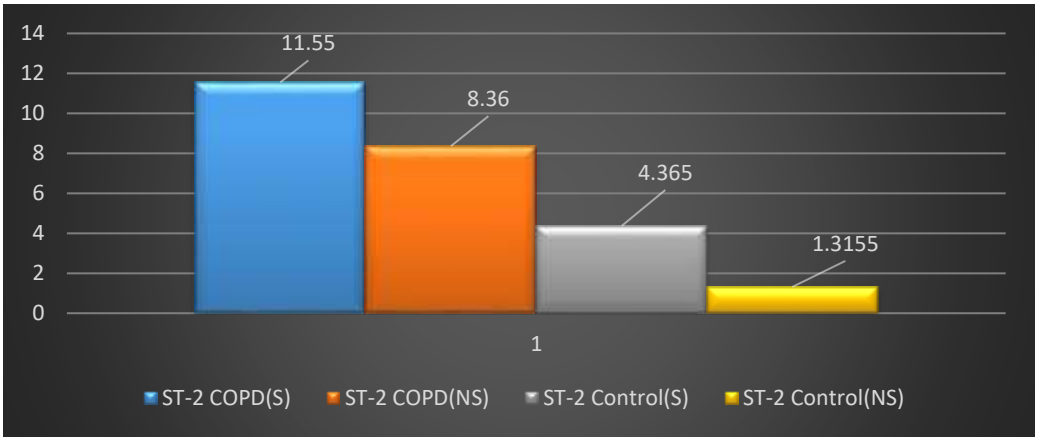

Figure 4: Comparison of ST-2(ng/ml) in Smoker and non-smoker COPD and control groups

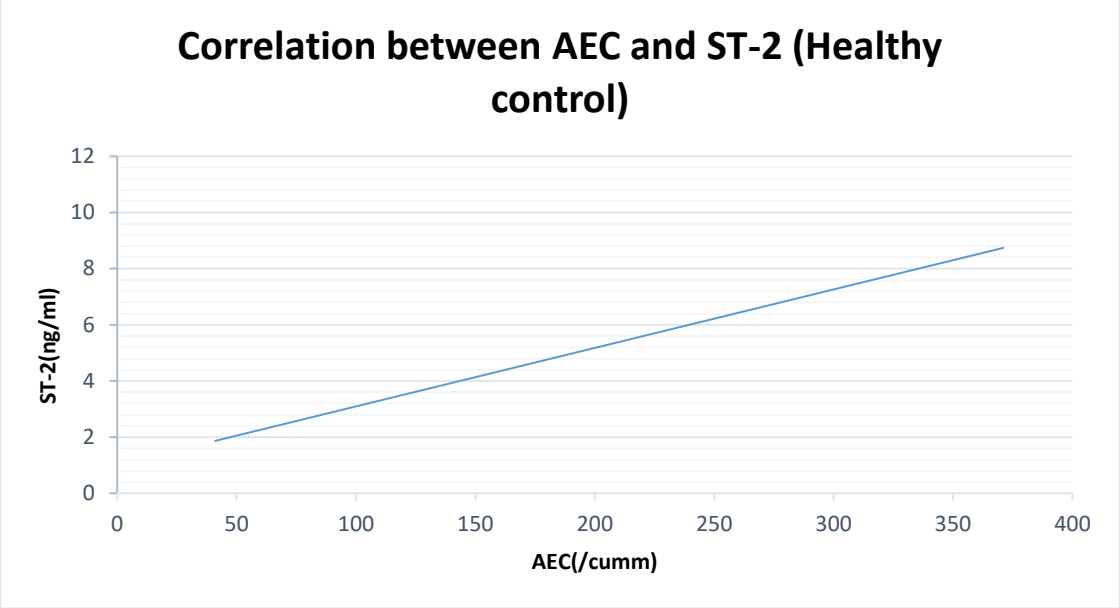

Figure 5: Correlation between absolute eosinophil count (/cumm) and ST-2 (ng/ml) in healthy control

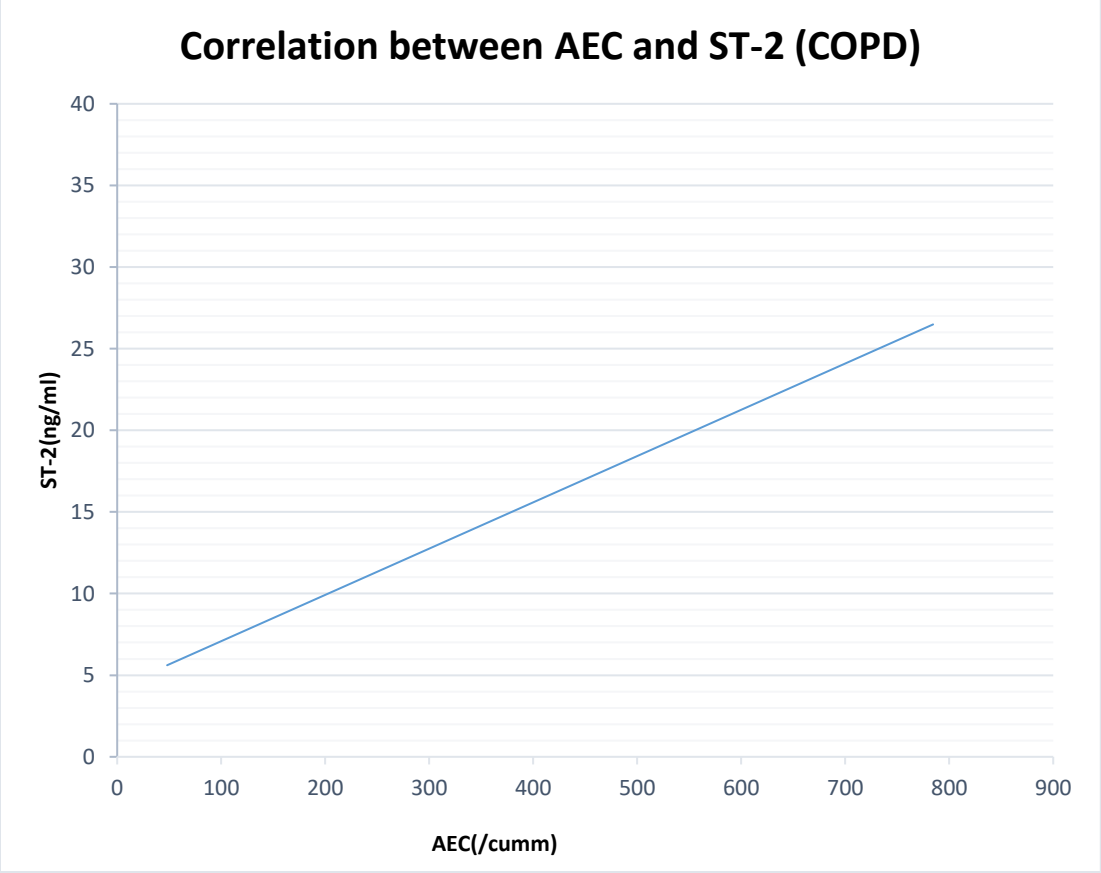

Figure 6: Correlation between absolute eosinophil count (/cumm) and ST-2 (ng/ml) in COPD patients

The present study addressed the effect of chronic trigger factors (such as smoking and others) in eosinophilic inflammation in COPD patients. The mean and standard deviation value for ST-2 in COPD patients is 10.72±7.44 ng/ml and in control group is 3.57±2.28 ng/ml, statistically significant($p < 0.0001$). The mean and standard deviation value for absolute eosinophil count in COPD patients is 225.73±146.65 /cumm and in control group is 123±57.48 /cumm, statistically significant ($p < 0.0001$). Xia J, (2014) et al also found significant ($P < 0.001$) difference between COPD patient and control subjects ST-2 level in plasma, which is similar to our findings[3]. Jiang M (2019) et al, Yuan Z (2016) and Gabryelska A (2019) et al also quoted similar findings in COPD patients[5, 8,14]. Tworek D (2018) et al also had similar finding in their study. They found increased number of ST-2 in sputum of COPD patients with airway eosinophilia ($p < 0.001$). The serum level of sST-2 was also increased ($p < 0.05$) in COPD patients as compared to healthy control. They also found overexpression of ST-2 mRNA ($p < 0.01$) in sputum of eosinophilic phenotype of COPD patients[15]. Increased eosinophil count in chronic respiratory disease was also reported by Donovan C. (2020) et al. They also reported reduced blood eosinophil in asthmatic patients after treatment with anti-IL-33 monoclonal antibody (Etokimab (ANB020) and this antibody is at phase 2 clinical trials for eosinophilic asthma. Furthermore, they quoted that in eosinophilic COPD phenotype, anti-IL-33 treatment could be beneficial[16]. In a study conducted by Allinne J (2019) et al in a model of chronic airway inflammation, the IL-33 blockage normalized persisting and exacerbating eosinophilic inflammation along with ST21CD41 T-cell infiltration[4]. Zeng S. (2015) et al performed a study in a mice model ,which showed a increased ST2 mRNA expression following intranasal infection with Respiratory syncytial virus (RSV) and then they used anti-ST2 monoclonal antibody to block ST-2 signalling and then found that RSV induced lung histopathology could be inhibited by anti-ST2 therapy and the AEC was also reduced after using anti-ST2 monoclonal antibody[17]. Jayalatha AS et al (2021) gave a confirmation of ST2 action as a decoy receptor by IL-33 sequestering in asthma and Yagami A. (2022) et al noted an increased expression of ST2L and sST2 in airway epithelial cells of lung tissue obtained from asthma patients. Asthma and COPD share a similar pathway of inflammation[18,19].

To further explore our findings, we compared ST-2 and AEC between COPD smokers and non-smokers and between healthy control smokers and non-smokers. Our results showed a statistically highly significant difference among ST-2 and AEC ($p<0.0001$) in a control smoker versus non-smokers group but not in COPD smokers versus non-smokers in AEC ($p=0.0588$) and ST-2 level($P=0.021$). Tworek D (2018) et al found overexpression of ST-2 mRNA in sputum cells from healthy smokers and COPD subjects compared with healthy non-smokers but in blood eosinophil count , no significant difference was recorded between COPD patients, healthy non-smokers and asthmatic subjects., which is similar to our findings .They also found increased EBC (exhaled breath condensate) absolute eosinophil count in asthma to healthy non-smokers (($p < 0.05$) and increased sST-2 expression in COPD ($p< 0.05$) patients as compared to healthy smokers[15]. Yuan Z(2016) noted twice higher level of serum ST2 in slight and moderate COPD patients as compared to healthy smokers[8].

The correlation analysis of AEC with ST-2 shows a positive correlation. Similar findings was reported by Tworek D (2018) et al who found a significant correlation between sST2, ST2 mRNA and sputum eosinophil content. In a study conducted by Yuan Z (2016) IL-13 genes were highly correlate with IL-33/ST-2 signal and IL-13 is a major cytokine involved in eosinophilic airway inflammation via IL-33/ST-2 pathway involvement. Kim SW (2017) also had similar findings with IL-33 and eosinophil count correlation and ST-2 is a decoy receptor for IL-33 [20] . A positive correlation in our study support the hypothesis that IL-33/ST-2 may be involved in development

of eosinophilic inflammation in COPD and by using antiIL-33/ST2 therapies, the eosinophilic inflammation in COPD may be reduced or inhibited and we propose that IL-33/ST-2 pathway can be a therapeutic indicator of COPD.

Conclusion:

This study confirms that there are increased serum ST-2 and AEC level and a positive correlation between AEC and ST2 in both COPD patients and healthy control. Thus it may be concluded that anti-ST2 therapy may be beneficial for COPD patients with eosinophilic inflammation to reduce the rate of exacerbation and death in COPD patients. However, to the best of our knowledge, this is first research conducted in India in COPD patients for the estimation of serum ST-2 levels and its correlation with AEC and these findings need randomized control trials.

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