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To cite this article before publication: Sanjeet Krishna Shrestha *et al* 2017 *J. Breath Res.* in press <https://doi.org/10.1088/1752-7163/aa95da>

Manuscript version: Accepted Manuscript

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Relationship between total serum immunoglobulin E levels, fractional exhaled breath Nitric Oxide levels and absolute blood eosinophil counts in atopic and non-atopic asthma: a controlled comparative study.

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No conflict of Interest

2. Respiratory and Occupational Practice

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INTRODUCTION

Asthma is a major non communicable respiratory disease with 235 million people suffering from it and 383 thousand deaths in 2015. Burden is prevalent in high as well as low income countries with higher mortality is lower income setting. This is usually attributable to inappropriate case identification and treatment. Nepal also suffers from huge burden of asthma.¹ Annual mortality due to asthma in Nepal was 11.8 per 100,000 person in 2013, with an average decline of 1.4% per year from 1990 to 2013. The disability-adjusted life years (DALY) though declining, is still 407.1 per 100,000 and mortality appears to increase with older population.² The prevalence of asthma in school going adolescents is moderate with values around 8.9% in Nepal.³

Asthma has several phenotypes. However, both atopic and non-atopic asthma is associated with airway inflammation. Association of asthma with immunoglobulin E (IgE) in serum has been known for a long time.⁴ Asthma is associated independently with total serum IgE levels irrespective of a specific IgE levels.⁵ There is a strong correlation between presence of asthma and airway hyper-responsiveness with higher levels of total serum IgE levels.⁶

There are four inflammatory asthma phenotypes on sputum cell counts: eosinophilic, neutrophilic, mixed granulocytic and paucigranulocytic asthma. All will have increased sputum eosinophil count with higher blood absolute eosinophil counts (AEC) in eosinophilic asthma phenotype.⁷ There is also a significant positive correlation between AEC, sputum eosinophil count and Total serum IgE levels.⁸

Fractional exhaled breath nitric oxide levels ($F_{E}NO$) has been utilized as a biomarker in asthma which indicates eosinophilic asthma.^{9, 10} $F_{E}NO_{50}$ is a cost efficient and easy to perform test which is available in few centres in Nepal. However, serum IgE level estimation is not easily available and is expensive when it is available. Sputum eosinophil counts are

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very rarely performed in Nepal. Hence, this study tried exploring the positive relation between all these investigations with the objective that $F_{E}NO$ can represent all these expensive and inaccessible tests.

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METHODS

This was a prospective unpaired investigative controlled trial conducted in randomly selected populations. The study group was designed to include 314 subjects with asthma group comprising 157 individuals and control group comprising 157 individuals. **Simple random sampling was done and the study was not blinded.**

The asthma group included subjects with diagnosed bronchial asthma as per Global Initiative for Asthma (GINA) guidelines with symptoms of wheeze, cough, chest tightness and breathlessness with documented variability of lung functions with spirometry. It included age population from 15 years to 80 years (In Nepal any patient at or above 15 years are referred to adult clinic). Patients were further subdivided into two categories: Atopic asthma, with history suggestive of any allergic phenomenon including rhinitis and eczema and Non-atopic asthma, with no history suggestive of allergic phenomenon. All groups of asthma populations were included in the study: mild, moderate or severe asthma depending on the treatment they are receiving. However, depending on the clinical control categorization of asthma, only partially controlled and uncontrolled asthma were included. This category included symptomatic asthma population. All the subjects were either non-smokers or ex-smokers (at least one year of abstinence from smoking) which was determined by simple questionnaire for smoking behaviour.¹¹

The healthy control arm of the study population was randomly selected. All controls were free from any respiratory or non-respiratory complaints. They did not have any of the four primary respiratory complaints as stated by GINA guidelines and were normal in their spirometry examination. Baseline chest radiograph were also normal in these subset. All the healthy participants were non-smokers and did not consume alcohol.

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Anyone below 15 years and above 80 years was excluded from the study. Pregnant ladies were also excluded from the study. All asymptomatic asthma population, which included well controlled asthma group were excluded from the study. All current smokers with or without asthma were excluded from the study. All patients with any other chronic respiratory or non-respiratory illnesses including bronchiectasis, chronic obstructive airway disease, pulmonary hypertension, rheumatological disease etc. were excluded from the study. Patients in any form of immune-compromised clinical conditions including chronic kidney disease, diabetes mellitus, use of oral immunosuppressive agents and use of corticosteroids were excluded from the study.

All the participants were explained about the study design and were asked for informed written consent. Detailed clinical history and examinations were done. Study subjects underwent $F_{E}NO_{50}$ breath analysis, Spirometry examination for lung functions, Serum estimation of total IgE levels and AEC.

Breath analysis for $F_{E}NO_{50}$ was done with point of care handheld single breath analyser BedFont NO breath device. The device is a gas analyser with electro-chemical sensor technology. This device is currently registered at England and Wales under Bedfont Scientific limited. The software with in the system is designed to detect low levels of nitric oxide in breath with good accuracy.¹² Food, beverages and exercise were avoided before the test.¹³ Detailed verbal explanations on how to perform the test were given to the patient followed by video demonstration of the breath analysis.¹⁴

After the complete exhalation of the breath subjects were asked to take a deep breath. Then with $F_{E}NO_{50}$ machine in hand, subjects were asked to exhale through the disposable mouth piece gradually into the device at a constant rate of 50ml/s as indicate by the metal ball at the

level of a white line on the device. The totals of three measurements were taken from each subject and the average values were recoded as the $F_{E}NO_{50}$ in parts per billion (ppb).

Lung functions were measured as a part of inclusion criteria to prove the presence and absence of airflow limitation, with rotating vane spirometer registered under Care Fusion as SpiroUSB device.¹⁵ It is a USB cable device attached to a desktop via USB port with accurate measurement of the forced vital capacity manoeuvre. The spirometry variables measured were: FEV1, FVC, FEV1/FVC and reversibility was measured after 15 minutes of inhalation of salbutamol 200ug via metered dose Inhaler device.¹⁶

The 5 ml of blood sample was drawn from each patient at time of enrolment into the study. The estimation of total IgE level was done with Chemi-luminescence Immuno-Assay (CLIA), which uses acridinium ester as the chemiluminescent label. It was done with ADVIA Centaur XP Immunoassay System. The level was expressed in IU/ml.¹⁷

AEC was done through eosinophil counting reagent in improved Neubauer Chamber (Teife Depth Profondeur) from LoLaboroptik.¹⁸

Outcome and variables

The study included three primary outcome variables all in continuous scale: Total Serum IgE level expressed in IU/ml, three measurements of $F_{E}NO_{50}$ level in breath expressed in ppb and its average and AEC in count/uL. Primary objective of the study was to find whether there was no difference between all three variables between asthmatics and non-asthmatics. The objective was to identify variable with a better sensitivity and specificity profile to identify asthma and atopy. This study also explored the possibility of difference of all three variables between atopic and non-atopic asthma. The correlation between all three variables between

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normal and asthma group and between atopic and non-atopic asthma group was also explored.

Statistical Analysis

The calculated sample size in each group was 157. So the samples size of the entire population was 314. All the preliminary data tabulation was done in Microsoft Word 2010 and data analysis was done using IBM SPSS Statistics Version 20. The Normality of data was checked using both Shapiro-Wilk and Kolmogorov-Smirnov test. Normally distributed demographic parameters were expressed in mean and standard deviations. Primary variables were identified by descriptive statistics to be skewed and distribution was non-normal. Expression of the data was hence done in terms of geometric means, median and standard deviation. Spread of the measured variables was expressed in 25th to 75th percentile and variance. Non-parametric test were used to identify correlation between the measured variables between the independent groups. Log transformation of the data was done to correct the effect of skewedness for data analysis. With the confidence interval of 95%, the statistical significant difference was defined with p value less than 0.05.

Ethical Justification

Ethical clearance for the study was taken from Institutional Review and Ethics committee of Kathmandu Medical College Teaching Hospital (KMCTH). Informed written consent was taken from each participant.

RESULTS

Total of 314 subjects were randomly selected for the study during the study period of August 2016 to May 2017. The study included 157 diagnosed cases of asthma with or without history of atopy and 157 normal healthy adults. On the week of enrolment all subjects underwent four investigations after detail history: $F_E NO_{50}$, AEC, IgE and Spirometry.

Observed measurements for all primary parameters were found to be in non-normal distribution. Hence data sets were expressed as geometric means, median, interquartile range and 25th to 75th percentiles. The statistical comparisons were done with log transformed data set. Detailed demographic characteristics of the study groups are given in table 1.

Table 1 Demographic characteristics of the study

Parameter	Asthma Group(n=150)			Control Group(n=150)			P Value
	Minimum	Mean	Maximum	Minimum	Mean	Maximum	
Age (yr)	15	32.37±11	74	21	31.8±9.8	61	0.376
Sex(M/F)		67/90			50/107		0.042
Height(cm)	138	160.6±7.55	180	140	158.6±7.6	182	0.734
Weight	30	60.58±10.69	92	45	62.96±8.11	85	0.06
BMI	14.53	23.48±3.93	36.85	17.30	25.10±3.28	33.77	0.178
	Atopic Asthma Group			Non-Atopic Asthma Group			
Age	15	33.67±11.38	74	15	32.4205	70	0.528
Sex(M/F)		39/30			26/62		<0.001
Height(cm)	139	162±7.37	180.00	138	159.59±7.55	180	0.051
Weight	33	60.56±10.93	80	30	60.60±10.58	92	0.983
BMI	15	23.04±3.81	30.44	15.79	23.79±3.99	36.85	0.251

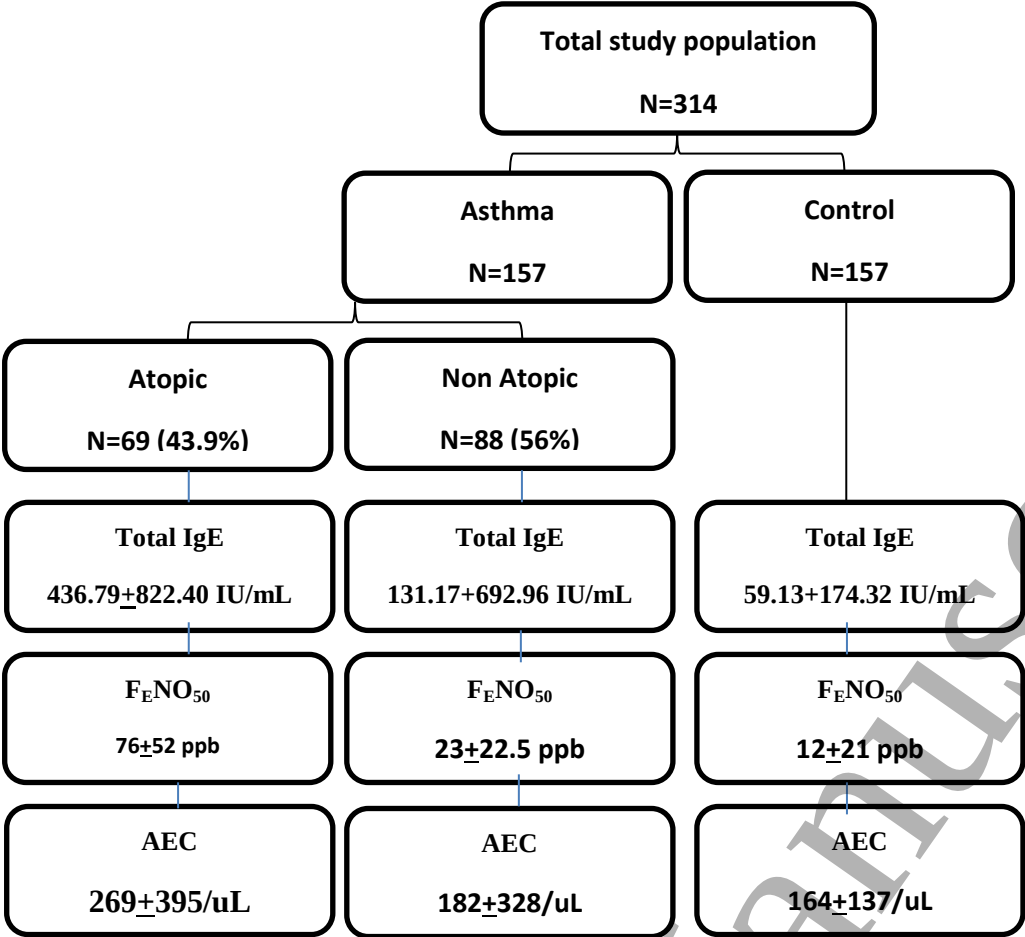


Figure 1 CONSORT flow diagram for the number of participants and primary outcomes.

Total IgE Levels

The geometric mean of total serum IgE level for the asthma group was 221.07 ± 774.55 IU/mL with median of 277. The values ranged from 2.3 to 3310 IU/mL. The 25th percentile was 61 IU/mL and 75th percentile was 948.8 IU/mL. The interquartile range was 891.63 IU/mL.

Similarly, the geometric mean of total serum IgE level for the control group was 59.13 ± 174.32 IU/mL with median of 63.2 IU/mL. The values ranged from 60 to 1124.5 IU/mL. The 25th percentile was 17.8 IU/mL and 75th percentile was 200 IU/mL. The interquartile range was 182.35 IU/mL.

The geometric mean of total serum IgE level for the Atopic asthma group was 436.79 ± 822.40 IU/mL with median of 618.95 IU/mL. The values ranged from 14 to 3310 IU/mL. The 25th percentile was 232.3 IU/mL and 75th percentile was 1180.2 IU/mL. The interquartile range was 956.5 IU/mL. Similarly, the geometric mean of total serum IgE level for the non-atopic asthma group was 131.17 ± 692.96 IU/mL with median of 174 IU/mL. The values ranged from 2.3 to 3000 IU/mL. The 25th percentile was 37.40 IU/mL and 75th percentile was 456.85 IU/mL. The interquartile range was 421.88 IU/mL.

There was statistically significant difference between the levels of total IgE in asthma group as compared to the control group ($p < 0.001$) with higher values recorded in the former. There was also a statistically significant difference between the levels of total IgE in Atopic asthma group as compared to the Non-atopic asthma group ($p < 0.001$) with higher values in the former. There was statistical difference identified between atopic asthma group and the control group ($p < 0.001$). Similarly there was statistical difference between non-atopic asthma group and the control group ($p < 0.05$).

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There was no statistical correlation identified between increasing age and levels of IgE in the entire study population ($p=0.613$). Similarly, there was no statistical correlation identified between age and levels of IgE in controls ($p=0.172$) and asthma group ($p=0.130$). Non-linear relationship was identified between these two variables (age and IgE levels). In the entire study population there was significantly higher IgE levels in males as compared to females ($p=0.001$). This trend continued with cases ($p=0.0230$) and controls ($p=0.014$).

Sensitivity of total IgE levels to detect asthma was only 57.3% with specificity of 73.3% with average cut off value of 200 IU/mL. Sensitivity of total IgE levels to detect atopy in asthma was 80.6% with specificity of 59.8% with average cut off value of 200 IU/mL.

Absolute eosinophil count

In the asthma group, the geometric mean for AEC was 214 ± 360 /uL with median of 220/uL. The values ranged from 5 to 2610/uL. The 25th percentile was 120/uL and 75th percentile was 440/uL. The interquartile range was 320/uL. In the control group the geometric mean of AEC was 164 ± 137 /uL with median of 160/uL. The values ranged from 20 to 900/uL. The 25th percentile was 120 and 75th percentile was 220/uL. The interquartile range was 100/uL.

In atopic asthma group the geometric mean of AEC was 269 ± 395 /uL with median of 257.5/uL. The values ranged from 5 to 2610/uL. The 25th percentile was 160 and 75th percentile was 500/uL. The interquartile range was 355/uL. The geometric mean of AEC for the non-atopic asthma group was 182 ± 328 /uL with median of 180/uL. The values ranged from 19 to 2225/uL. The 25th percentile was 103 and 75th percentile was 395/uL. The interquartile range was 295/uL.

There was statistically significant difference between AEC in asthma group as compared to the control group ($p=0.002$) with higher values in the asthma group. There was statistically

significant difference between the levels of AEC in Atopic asthma group as compared to the non-atopic asthma group ($p=0.008$). The values were statistically higher in the atopic asthma group.

Sensitivity of AEC to detect asthma was only 33.3% with specificity of 88% with average cut off value of 350/uL. Sensitivity of AEC to detect atopy in asthma was very low (41.9%) with specificity of 72.4% with same cut off value as above.

FeNO Values

The data was non-normally distributed. The geometric mean of $F_{E}NO_{50}$ for the asthma group was 37 ± 49 ppb with median of 36 ppb. The values ranged from 4 to 235. The 25th percentile was 18 and 75th percentile was 80. The interquartile range was 62 ppb. Similarly, in the control group the geometric mean of $F_{E}NO_{50}$ was 12 ± 21 ppb with median of 13 ppb. The values ranged from 1 to 149 ppb. The 25th percentile was 8 ppb and 75th percentile was 19 ppb. The interquartile range was 11 ppb.

The geometric mean of $F_{E}NO_{50}$ for the atopic asthma group was 76 ± 52 ppb with median of 82 ppb. The values ranged from 11 to 235 ppb. The 25th percentile was 58 and 75th percentile was 128 ppb. The interquartile range was 72 ppb. The geometric mean of $F_{E}NO_{50}$ for the non-atopic asthma group was 23 ± 22.5 ppb with median of 24 ppb. The values ranged from 4 to 114 ppb. The 25th percentile was 15 and 75th percentile was 36 ppb. The interquartile range was 21 ppb.

There was statistically significant difference between $F_{E}NO_{50}$ in asthma group as compared to the control group ($p<0.001$). Atopic asthma group as compared to the non-atopic asthma group showed statistically higher $F_{E}NO_{50}$ measurements ($p<0.001$).

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Sensitivity of $F_{E}NO_{50}$ levels to detect asthma was only 42.7% with specificity of 96.7% with average cut off value of 50 ppb. However, Sensitivity of $F_{E}NO_{50}$ levels to detect asthma rose to 65.3% with specificity of 91.3% if cut off value was reduced to 25 ppb. Sensitivity of $F_{E}NO_{50}$ levels to detect atopy in asthma was 82.3% with specificity of 85.1% with average cut off value of 50 ppb. However, sensitivity rose to 90.3% and specificity 51.7% with cut off values at 25 ppb.

Correlation between IgE, FeNO and AEC

Non parametric correlations between IgE level and $F_{E}NO_{50}$ showed positive moderate correlation with spearman's rho of 0.461. Parametric correlation with log transformed data showed positive moderate correlation with Pearson's correlation coefficient of 0.470. Similar correlation between IgE level and AEC showed positive moderate correlation with spearman's rho of 0.315. Parametric correlation with log transformed data showed positive moderate correlation with Pearson's correlation coefficient of 0.315.

In the study groups, non-parametric correlation between $F_{E}NO_{50}$ and AEC was positive with small degree of correlation (spearman's rho of 0.299). Parametric correlation with log transformed data showed positive small correlation with Pearson's correlation coefficient of 0.294 between $F_{E}NO_{50}$ and AEC. This trend was similar in asthma group with significant correlation between AEC and $F_{E}NO_{50}$ ($p<0.001$). However such correlation was not significant in control group ($p=0.205$).

Table 2 Immunoglobulin E (IgE), Absolute Eosinophil Count (AEC) and Fractional Exhaled Nitric Oxide ($F_{E}NO_{50}$) levels in the study groups.

	Asthma Group GeoMean $\pm$ SD (Median)	Control Group GeoMean $\pm$ SD (Median)	P-Value
IgE level (IU/mL)	221.07 $\pm$ 774.55 (277)	59.13 $\pm$ 174.32 (63.2)	P<0.001
	Atopic Asthma	Non Atopic Asthma	
IgE level (IU/mL)	436.79 $\pm$ 822.40 (618.95)	131.17 $\pm$ 692.96 (174)	P<0.001
	Asthma Group	Control Group	
AEC (/uL)	214 $\pm$ 360 (220)	164 $\pm$ 137 (160)	P=0.002
	Atopic Asthma	Non Atopic Asthma	
AEC (/uL)	269 $\pm$ 395 (257.5)	182 $\pm$ 328 (180)	P=0.008
	Asthma Group	Control Group	
$F_{E}NO_{50}$ Level (ppb)	37 $\pm$ 49 (36)	12 $\pm$ 21 (13)	P<0.001
	Atopic Asthma	Non Atopic Asthma	
FeNO Level (ppb)	76 $\pm$ 52 (82)	23 $\pm$ 22.5 (24)	P<0.001

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DISCUSSION

Predominant association of asthma with IgE mediated inflammation has been the main stay of our understanding of allergic origin of asthma.⁴ Both specific and total IgE levels play major part in upstream cascade in asthma inflammation.¹⁹ Values of IgE have been extensively evaluated in different study population. Mean IgE levels in Bangalore, India was 151.95 IU/ml in normal subjects and 1045.32 IU/ml in severe asthma. There is also a trend of increasing levels of IgE with ‘GINA severity of asthma’ indicating its role in pathogenesis of asthma and its severity.²⁰ In our study geometric mean was 221.07 ± 774.55 IU/mL in asthma group and control group was 59.13 ± 174.32 IU/mL. Values observed were significantly lower than those mentioned in above study but there was statistical difference between the two groups.

Higher the IgE level chances of asthma being more severe is highly probable and higher IgE is known to be characteristically associated with moderate to severe asthma. The high level of IgE , persistent mucous secretion and cough have been identified as a distinct phenotype.²¹ In our study asthma control status (partial control to poor control) had significant correlation with the levels of IgE. Poorly controlled asthma had higher levels of total IgE. Highest IgE level recorded in present study was 3310 IU/mL. Total of 36 subjects had IgE levels at or above 1000 IU/mL in the entire study population. Among these subjects 34 were in asthma group. Only two subjects were in control group. If we presume upper limit of IgE as 300 IU/mL then there were 92 subjects with levels ≥ 300 IU/mL among which 19 were controls. This states that not all subjects with high IgE had asthma. Higher IgE may even be present in healthy adults.^{22, 23}

Furthermore gradual longitudinal increase in IgE level is shown to be associated with increased frequency of exacerbation, higher steps of treatment, use of oral corticosteroids and

uncontrolled asthma. Reverse is true for asthma on treatment with longitudinal decrease of IgE.²⁴ The present study did not include longitudinal follow ups.

Large population based studies have shown different trends of total and specific IgE levels. Mara De Amici et al. concluded in their paper that there is increase of total IgE levels with increasing age but decrease of allergen specific IgE levels.²⁵ However, previous researchers have also shown decrease (negative correlation) of total and specific IgE levels with age with allergic rhinitis and asthma.²⁶ In our study there was no significant correlation of total IgE level with age both in asthma and control groups. There was also no correlation between age and IgE levels in atopic and non-atopic subjects.

Even atopic individuals can have a longitudinal decline of IgE level with age; more so with females as compared to males. The IgE level tends to be higher in atopic males as compared to atopic females after the age of 35.²⁷ In the present study, total IgE levels were higher in males as compared to females which were statistically significant in the entire study group and also with in asthma group. However total IgE levels were not statistically different between males and females in the control group ($p=0.369$). Higher levels of total IgE have been defined in atopic individuals suffering from asthma, allergic rhinitis and urticaria.^{28, 29} Sex ratio matching was done at the onset of the study to rectify this bias, (table 1) however sex ratio matching could not be established between atopic and non-atopic groups.

All the participants denied consumption of alcohol. Alcohol consumption can be a confounding factor in the measurement of total IgE levels both in atopic and non-atopic individuals. Total IgE levels increase with increasing levels of alcohol consumption (greater than 70g/day). Hence, even alcohol consumption in moderation tends to elevate total IgE levels.^{30, 31} We excluded alcohol consumers in the study to check this bias. We did not further evaluate the effect of alcohol in IgE level in our study groups.

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Initial studies of Gerard and colleagues stated that habit of smoking is associated with elevated levels of IgE.³² The relationship of IgE levels with smoking was further clarified by Burrows et al. stating that smoking can induce immunological adverse effect with elevated IgE which increases with age.³³ Some studies have quoted smoking to be associated with increase in the levels of IgE particularly for males.³⁴ It is for these evidences that the present study excluded all the smokers from the study in both the asthma and non-asthma group.

Estimation of IgE levels in asthma for identification of phenotype and anagement of asthma is now a routine practice. Several prominent guidelines and papers including GINA guideline and British guideline have included IgE level as an important part in the investigation and management of asthma. IgE levels have taken further importance due to the utility of Anti-IgE measures in management of allergic asthma and difficult to treat asthma.^{35, 36}

FENO has been added as a very important inflammatory biomarker both for the diagnosis and management of asthma. Levels that are higher than 50 ppb in non-smokers are associated with eosinophilic airway inflammation.³⁵ Its association however is modest and requires careful interpretation. Probably the most important aspect of F_ENO₅₀ is the ease of use and ready availability.¹⁰ There has been research regarding association of elevated FENO levels with atopy as compared to non- atopic individuals.³⁷ It's a biomarker suggesting atopy in asthma and has a strong association with allergic asthma phenotype.³⁸ In the present study also there was significant difference in F_ENO₅₀ levels between asthma and control groups with higher values in the former. The levels were also significantly higher in atopic asthma as compared to non-atopic asthma. F_ENO₅₀ had a better sensitivity and specificity profile to identify asthma as compared to total IgE level and AEC. In fact it had a better sensitivity and specificity profile to detect atopy in asthma as compared to total IgE level.

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5 and highest peak observed in teenagers. The literature supports the notion of gradual increase
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7 of F_ENO during childhood development and adolescence with sustained levels during adult
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9 life which tends to increase after sixth decade till eighth.^{39, 40} There was no statistical
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11 correlation between age and level of F_ENO in both asthma and non-asthma group, in our
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18 There are several factors that confound the use of F_ENO in daily clinical practice. Any form
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20 of atopy and eosinophilic reaction can cause elevation of F_ENO levels. Higher levels are seen
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22 with eosinophilic asthma/Atopic asthma, eosinophilic bronchitis, eosinophilic inflammation
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24 in chronic obstructive pulmonary disease and eosinophilic lung disease.^{9, 10, 35} In the present
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26 study weak correlation was identified between F_ENO and AEC. Spuriously low levels of
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28 F_ENO can also pose problem especially when asthmatics continue to smoke or when they are
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30 obese. Normal F_ENO levels have been associated with controlled asthma, neutrophilic, non-
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32 eosinophilic asthma or severe bronchoconstriction in asthma.^{9,10, 35,41, 42} Reverse is also found
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34 to be the trend with F_ENO levels increasing after the smoking cessation.⁴³ An Official ATS
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36 guidelines on technical aspects of F_ENO and Interpretation of F_ENO levels have been
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38 published and are considered the standards of current diagnostic utility of F_ENO.¹⁰
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45 Increase in AEC occurs often in asthma as eosinophilic inflammation is a key pathogenic
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47 pathway in airway inflammation. There appears to be a positive association between
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49 increasing AEC with increasing clinical severity of asthma.⁴⁴ Hence, AEC is now considered
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51 a useful biomarker in asthma to identify the eosinophilic phenotype and is a common target
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53 in newer treatment modalities.⁴⁵ AEC has also been found be a useful marker to detect the
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55 nasal and bronchial allergy and its severity.^{46, 47}
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Targeting sputum and AEC has been the mainstay of recent advances in targeted therapy in allergic eosinophilic asthma, which is common in adult onset asthma.⁴⁸ Since sputum eosinophil counts are not being done routinely in Nepal, AEC in peripheral blood, IgE and F_ENO can act as alternative markers of eosinophilic inflammation in asthma. However these surrogate markers have moderate diagnostic accuracy.⁴⁹

Significant positive correlation has been identified in array of clinical research both in paediatric and adult population between IgE levels, F_ENO levels and AEC.^{50, 51, 52} Some studies have shown weak correlation between IgE levels and F_ENO levels stating that higher value of one variable may not necessarily result in higher value of the other one.⁵¹ and others have shown total IgE having weak correlation with AEC.⁵² Moderate level of correlation has also been observed in literature between F_ENO₅₀ and AEC.⁵³ Our study also found moderate level of correlation between these variables. Such positive correlation has been found in adults with refractory asthma also.⁵⁴ Positive associations have been defined in various research papers between AEC and Total serum IgE levels.^{55, 56}

Limitations

The study was an un-blinded study designed as an open recruitment with patients being enrolled as they arrived to the respiratory clinic and when they qualify all the inclusion and exclusion criteria. During the recruitment higher numbers of female participants were enrolled as compared to males. There was no statistical difference between sex ratios in asthma and non-groups but within asthma group, atopic and non-atopic groups had statistical difference between sex ratio. This could have introduced bias into the comparison. During this study subjects were not asked to follow up routinely for the study purpose. Hence, we are not able to state longitudinal effects on IgE, AEC and F_ENO₅₀.

CONCLUSION

All three variables: total serum IgE, $F_{E}NO_{50}$ and AEC levels were higher in asthma group as compared to controls. Atopic phenotypes had higher levels of all three variables as compared to non-atopic phenotypes. There was moderate level of correlation between IgE with $F_{E}NO_{50}$ and AEC. A weak correlation was identified between $F_{E}NO_{50}$ and AEC. Sensitivity and specificity profile was better for $F_{E}NO_{50}$ to identify asthma in the entire study group as compared to IgE and AEC. Sensitivity and specificity was also higher for $F_{E}NO_{50}$ to predict Atopy among asthma group. Sensitivity of AEC was lowest among all study variables.

$F_{E}NO_{50}$ is less expensive in Nepal as compared to total IgE estimation. It is a more sensitive and specific tool for asthma management. Hence, $F_{E}NO_{50}$ can be a better surrogate for IgE level. $F_{E}NO_{50}$ and AEC are weakly correlated and may not represent each other independently.

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Acknowledgment

The authors wish to acknowledge the support shown by Nepal Med e.V. for this research for providing F_ENO₅₀ machine during the study period and facilitating in the estimation of serum total IgE level. Nepal Med e.V. is a non-governmental organization working for better health in Nepal, currently registered at Germany.

Accepted Manuscript

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