Assessment of Serum Activin A level and its value as a biomarker for predicting subsequent asthma in wheezy infants

1- Corresponding author and First author:
Heba Ahmed Said Mahmoud Raslan
Heba Raslan
Affiliation: Department of Pediatrics, Cairo University Children Hospitals, Faculty of Medicine, Cairo University, Cairo, Egypt
Email: hebaraslan@hotmail.com
Phone: 020 1005110001
2- Second author:
Mohammed Abdel Fattah Abdel Motey
Mohammed Abdel Motey
Affiliation: Department of Pediatrics, Cairo University Children Hospitals, Faculty of Medicine, Cairo University, Cairo, Egypt
Email: maf939@hotmail.com
Phone: 020 1005248803
3- Third author:
Nihal Mohamed El Rifai
Nihal El Rifai
Affiliation: Department of Pediatrics, Cairo University Children Hospitals, Faculty of Medicine, Cairo University, Cairo, Egypt
Email: nihal-r@hotmail.com
Phone: 020 1276333317
4- Fourth author:
Walaa Ahmed Mohamed Rabie
Walaa Rabie
Affiliations: Department of Clinical and chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt
Email: wl.rabie@gmail.com
Phone: 020 1227432769

ABSTRACT

Background: Activin A cytokine has grabbed attention regarding its role in inflammation and airway remodeling in asthma as it regulates airway inflammatory cells and airway remodeling. However, knowledge regarding its role in wheezy infants is limited.

Patients and Methods: The present study was designed as a case control study that included two groups. First group, 40 Egyptian infants less than 2 years referred to the allergy clinic with history of their first or second attack of wheezing with mean age 14.925 ± 5.942 months with male to female ratio of 1.35, and second group, their matched control group (n=40). After comprehensive clinical assessment, blood samples were taken from the patients in order to assess serum activin-A, IgE and CBC measurements.

Results: Patients had statistically significant higher activin A level compared to healthy controls (529.8 ± 358.4 vs 227.9 ± 63.4 pg/ml, $p<0.001$). Activin-A levels had a strong positive correlation with IgE levels ($r=0.760$) ($p<0.001$) and eosinophilic count ($r=0.758$) ($p<0.001$). Predictive ability of activin-A in discriminating wheezy infants from healthy controls showed that activin-A had high specificity of 95% and low sensitivity (55%). Mean serum activin A was significantly higher in patients who had history of recurrence of wheeze and hospital admission at follow-up. There were significant direct correlations between serum activin A and recurrence of wheezes, number of attacks and hospital admission within 6 months of the study at follow-up.

Conclusion: Serum activin A is significantly higher in wheezy infants compared to normal controls, and that it directly correlates with recurrence of wheeze, number of attacks and need for hospitalization at follow-up. Thus, increased serum activin A is postulated to be a potential predictor for future occurrence of asthma in wheezy infants.

Key words: Activin A- asthma- children- wheezy infants

Manuscript

Introduction and aim of the study

Activin A, a dimer of βA subunits, is a member of the transforming growth factor-β (TGFβ) superfamily. The importance of activin A as a major regulator of the inflammatory response is supported by its capacity to stimulate monocytes/macrophages to produce several inflammatory mediators including IL1B, TNF, IL6, nitric oxide, prostaglandin E2 and thromboxane**.** [1] Accumulating evidence indicates that activin A drives tissue fibrosis in a variety of organs and is implicated in airway remodeling. Activin A has been implicated in lung fibrosis via promotion of collagen and alpha smooth muscle activin synthesis and proliferation of human airway fibroblasts and smooth muscle. [2] The aim of this work is to assess serum activin A level in wheezy children and to evaluate its predictive value of asthma in infants with recurrent wheezing.

Patients and methods

40 Egyptian children less than 2 years referred to the allergy clinic of Pediatric Department, Japanese Pediatric Hospital, Cairo University with history of their first or second attack of wheezing during the period from April 2015 to June 2016 were included. In addition, 40 age and sex matched controls were included. Appropriate consent from patient's guardians and IRB approval was taken.

Infants during an acute attack of wheezy chest, history of prematurity, history of NICU admission or assistant ventilation were excluded. Additionally, infants on long term asthma controller medications, receiving systemic steroids within the 2 weeks preceding sampling or have other causes of elevated levels of IgE, for instances, parasitosis were excluded.

Detailed history of wheezing, previous hospital admission or medications as bronchodilators, history or manifestations of atopy were collected.

Blood samples were taken from the patients using wide-bore needle and withdrawn slowly from antecubital vein to avoid hemolysis of RBCs by careful venipuncture. These samples were divided into 2 aliquots, first aliquot was added to EDTA tube for CBC measurements and the other aliquot was added to a dry tube, allowed to be clotted for 30 minutes, and then separated by centrifugation at 4000 rpm for 5 minutes.

For determination of serum IgE antibodies, RIDASCREEN® Total IgE, an enzyme immunoassay, was used. Next, an anti-human IgE antibody conjugated with alkaline phosphatase is added and attaches to the bound human IgE antibody. Finally, a substrate is added and dephosphorylated by the alkaline phosphatase. The resulting color change from colorless to yellow is measured using a photometer at 405 nm and a reference wavelength of 620 nm. The results are expressed in IU/ml.

A reference curve was set up in order to evaluate the test. The average extinctions from the double determinations of the standards were plotted semi-logarithmically (x-log/y-lin presentation) as a function of the concentration (IU/ml) in a point-to-point presentation. The concentration of the IgE antibodies can be determined from the measured OD using the standard curve.

The standard values for total IgE levels as a function of age (IgE standard values, in IU/ml, as a function of age (95 % percentiles) is less than 2 for newborn, 40 in first year of life and 100 in 2nd year.

For estimation of serum Activin A, the assay employs a 3-step quantitative sandwich enzyme immunoassay technique. The capture antibody is biotinylated and bound to streptavidin-coated plates. The plates are washed and Assay Diluent, Standards, and samples are pipetted into the wells and any Activin A present is bound by the immobilized antibody. After washing away any unbound substances, an HRP-conjugate specific for the βA subunit is added to the wells. Following a wash to remove any unbound conjugate, a substrate solution is added to the wells

and color develops in proportion to the amount of Activin A bound. The color development is stopped and the intensity of the color is measured.

For calculation of the results, the readings of duplicate standard, control, and sample and subtract recorded against the average zero standard optical density. The optical density for the standards plotted versus the concentration of the standards on an appropriate curve. The data linearized by using log/log paper. Activin A concentration of each sample was determined by plotting the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, a vertical line extended to the x-axis and the corresponding Activin A concentration was recorded (figure 1). Normal serum level of Activin A is between 142 - 753 pg/mL, and normal plasma level of Activin A is between 111 - 680 pg/mL

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 25 (IBM Inc. USA) software. The following tests were used: Frequency distributions, percentage distributions, Means ± standard deviation, t-tests, chi-square test, Fisher's exact test, tests of correlation, receiver operating characteristic (ROC) test and logistic regression. P values less than 0.05 were considered significant. Confidence intervals (95% CI) were calculated when appropriate. Graphs were generated using Microsoft Excel 2007 (Microsoft Co. USA) and MedCalc version 15.8 (MedCalc Software, Ostend, Belgium).

Results:

This cases-control study included 40 Egyptian wheezy infants as cases and 40 age- and sex-matched healthy infants as controls. The age ranged from 3 to 24 months. The mean age was 14.93 ± 5.94 months in wheezy patients and 12.85±6.02 months in controls, with male/female ratio 1.35. The mean age of onset of wheezing was 3.95±2.05 months, occurring in winter in 70%. 70% had exposure to passive smoking. Food allergy was reported in 32.5% (n=13), mostly to dairy products (25%). Infections precipitated wheeze in 50% of cases. Family history for atopy was reported by 67.5%, with family history of asthma in 37.5%. Allergic rhinitis and eczema were the most common atopic manifestations (both of 32.5%).

The mean total leucocytic count (10.24±2.5 vs 8.32±1.29 10^3/cmm), platelets (354.1±94.7 vs 300.5±67.9 10^3/cmm) and absolute eosinophilic count (317±212 vs 132.3±33.7 /cmm) were significantly higher in cases compared to controls ($p<0.001$, $p=0.005$, $p<0.001$ respectively).

The mean serum total IgE was significantly higher in cases (63.8±98 IU/ml) compared to controls (8.17±6.00 IU/ml) ($P=0.001$). The mean serum total activin A was significantly higher in cases (529.8±358.4 pg/ml) compared to controls (227.9±63.4 pg/ml) ($P<0.001$). Regarding the diagnostic accuracy of serum activin A in wheezy infants using Receiver operating characteristic (ROC) curve for serum activin A, serum activin A had a low sensitivity of 55% and a high specificity of 95% in discriminating wheezy infants from normal controls. (AUC=0.750, $P<0.001$).

Patients were followed up for a period of 6 months. During follow up, 42.5 % (n=17) of cases suffered from recurrence of wheeze. The mean serum activin A was significantly higher in patients who had recurrence of wheeze at follow-up (899±219 pg/ml) compared to non-recurrence group (256.7±97 pg/ml) (P-value <0.001). Additionally, 8 cases (20%) required hospitalization during follow up with their mean serum activin A (1074±77 pg/ml) significantly higher than patients who had no history of hospital admission during follow-up (394±254 pg/ml) (P-value <0.001). There were significant direct correlations between serum activin A and recurrence of wheezes (0.852, P<0.001), number of attacks (0.889, P<0.001) and hospital admission (0.693, P<0.001) within the follow-up.

Discussion:

The present case control study was conducted on 40 Egyptian children less than 2 years referred to the allergy clinic with history of their first or second attack of wheezing. We assessed the level of serum activin A in wheezing patients and evaluated its predictive value of asthma in infants with recurrent wheezing.

Our study found that wheezy patients had statistically significant higher activin A level than healthy controls (529.8 ± 358.4 vs 227.9 ± 63.4 pg/ml, p<0.001). This result ties well with the previous literature. Samitas et al. reported significant increase in serum activin A in asthmatic patients compared to healthy controls.[3] This was not limited to activin A in serum only; activin A levels in bronchoalveolar lavage and its expression in epithelial and sub-epithelial inflammatory cells of asthmatic patients was significantly higher especially those with severe grade. Similarly, Papaporfyriou et al. found that asthmatic patients showed considerably higher sputum levels of activin A than healthy controls (76 (33–185) vs 29 (25–31) pg/ml, and that activin A level was significantly higher in bronchoalveolar lavage in asthmatic patients especially those with severe refractory form.[4] Higher levels of activin A were reported in serum of asthmatic patients compared to their healthy counterparts especially those who are not on glucocorticoid treatment.[5] Elevated activin A expression was reported in bronchial epithelial and subepithelial cells from biopsies of asthmatic patients, compared to healthy controls.[6]

Human rhinovirus is known for its association with childhood asthma risk.[7] Leigh et al. suggested that activin A plays a major role in this association.[8] High levels of activin A were detected during human rhinovirus infection, which attacks airway epithelial cells leading to early airway remodeling.[8]

The mechanism explaining increased activin A levels in asthmatic patients has been explored by studying CD4+ T cells that play an essential role in asthma.[9] Asthmatic patients not on glucocorticoid treatment, showed an increased activin A mRNA expression by CD4+ T cells more than healthy control subjects.[5] In this hypothesis, activin A has pro-inflammatory role through inducing mast cell activity and maturation as well as production of antibodies from B-cells.[10] On the other hand, Semitekolou et al. related the elevated activin A levels in asthmatic patients to its anti-inflammatory role trying to curb and neutralize the ongoing inflammation

during asthmatic attacks which is best seen in patients with severe asthmatic form through regulation of Th2-type allergic response.[6] In-vitro studies showed that activin A is allergen inducible and its level can be increased by increasing allergen exposure.[11]

We found that activin A levels had a strong positive correlation with IgE levels ($p<0.001$). Asthmatic patients demonstrate higher levels of IgE compared to healthy individuals as it plays an important role in the pathogenesis of atopic diseases.[12] Our study showed that asthmatic patients had significantly higher serum total IgE compared to controls. Similarly, Samitas et al. found that severe asthmatics had significantly higher serum total IgE compared to mild asthmatics and healthy controls.[3] The association between IgE levels and activin A has been discussed in previous studies; Cho et al**.** found that cross-linking of IgE on mast cells leads to a rapid increase of activin A mRNA expression.[13] Activin A increases antigen-specific IgE production, promotes the differentiation of macrophages into the M2 phenotype, and stimulates the maturation of mast cells.[14] Moreover, in-vivo studies showed that IgE levels of specific allergens decreased after neutralization of the circulating activin A.[15]

Eosinophils and its proinflammatory role in asthma has been well established before.[16] In our study, absolute eosinophilic count was significantly higher in cases compared to controls. In addition, activin A levels had a strong positive correlation with eosinophilic count ($p<0.001$). Our results are in agreement with Papaporfyriou et al. who found a significant correlation between activin A and eosinophils in sputum and in bronchoalveolar lavage fluid from asthmatic patients.[4] Similarly, Kelly et al. reported that activin A expression was directly correlated to eosinophil numbers.[17] On the other hand, an in vitro study found that follistatin, that acts as activin A antagonist**,** failed to reverse eosinophil recruitment and proliferation in airways.[15]

We calculated the receiver operating characteristic (ROC) curve for all recruited infants of the study. The area under the curve (AUC) was fairly acceptable (0.75), which means that serum activin A levels are capable of discriminating wheezy infants from healthy controls with a reasonable accuracy, with the best cutoff being levels above 340 pg/ml. Activin A showed high specificity of 95%; infants with normal or low activin A are unlikely to have wheezes or asthma (a good negative screening test). However, it showed modest sensitivity of 55%; infants with high activin A may or may not have wheezes or asthma (unreliable positive screening test). This was also demonstrated it its high negative predictive value (96.2%) and poor positive predictive value (47.9%).

We attempted to investigate the role of activin A levels in predicting infants with recurrent wheezing who might end up having asthma. Follow-up of studied children was carried away. Parents were inquired about history of recurrent wheezing, the number or wheezy episodes and history of admission to hospital or intensive care units (ICUs) in the six months following study inclusion. About 42.5% had history of recurrent wheezing, 37.5% of which had one or two episodes, while the remaining 5% had three or four episodes. Twenty percent of cases were hospitalized within 6 months follow-up due to wheezes, and only one patient (2.5%) needed ICU admission. upon analysis we found that mean serum activin A was significantly higher in

patients who had recurrence of wheeze compared to those who did not (899±219 versus 256.7±97 pg/ml, respectively, $p<0.001$). Furthermore, it was noted that mean serum activin A was significantly higher in patients who were hospitalized compared to those who were not hospitalized (1074±77 versus 394±254 pg/ml, respectively, $p<0.001$). Again, there were significant direct correlations between serum activin A and recurrence of wheezes, number of attacks and hospital admission within 6 months of the study. Although correlation does not necessarily verify causation, yet it guardedly proposes that elevated serum levels of activin A might be considered a prognostic indicator of developing persistent wheezing or asthma later in life, Further studies on a larger scale with serial measurements of activin A and longer duration of follow-up might help shed more light on this matter. Up to the authors' knowledge, this correlation was not reported before in infant wheezers in published literature.

Our study brings novel findings regarding levels of activin A in wheezy children below 2 years. In published literature, studying activin A levels was focused on adult asthmatic patients and in-vitro studies. To our knowledge, this is one of the few studies to investigate serum activin A levels in wheezy infants and compare it to healthy controls. Activin A has been proposed as a predictive biomarker in several clinical outcomes apart from asthma.[18,19] However, our study assessed the predictive ability of activin A in discriminating wheezy infants from healthy controls, where activin A had high specificity of 95% in discriminating wheezy infants from normal controls despite being not sensitive (55%).

Nevertheless, our study had its limitations; The sample size was relatively small, and the study had no multicenter design, so its results might not be generalized. We couldn't include pulmonary functions in our work due to young age of infants and other difficulties. Again, measurement of serum activin A was made once and serial measurements over time were not performed. The relationship of activin A with body mass index (BMI) and treatment administered were not evaluated in our study. To illustrate, Zhang and Liu highlighted the importance of BMI in affecting the levels of activin A among asthmatic patients and reported that increased serum level of activin A indicates its role in the pathogenesis of asthma, particularly in underweight and overweight patients but less in patients with normal BMI.[20]

Conclusion:

Serum activin A levels are significantly higher in wheezy infants compared to normal controls with significant direct correlation between serum activin A and recurrence of wheezes, number of attacks and hospital admission. Despite that serum activin A has low sensitivity in discriminating wheezy infants from normal controls, it's high specificity render it a valuable prognostic index for future asthma development in wheezy child.

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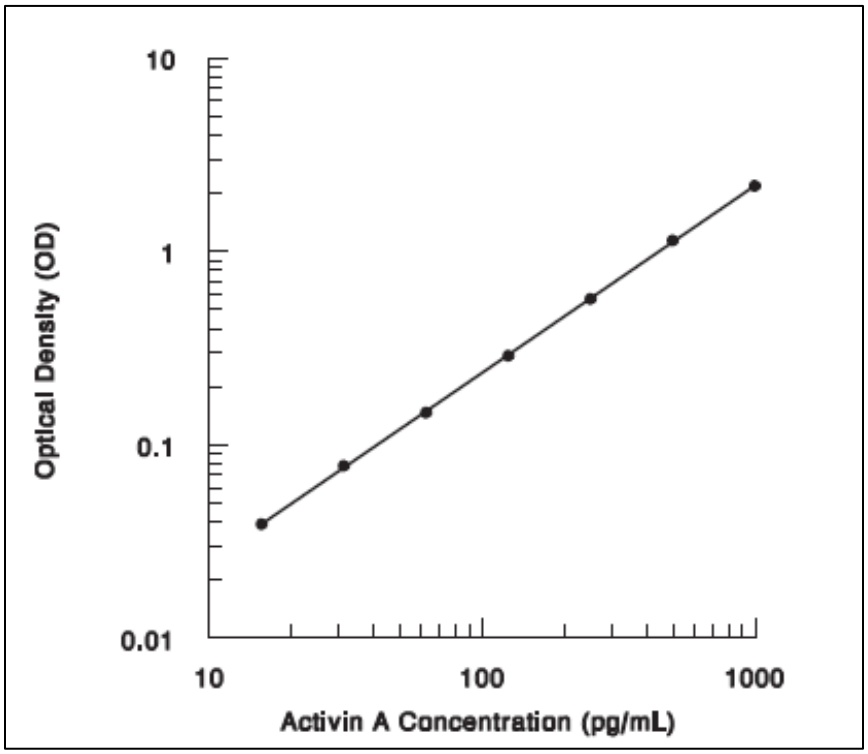
Figure legend

Figure 1: This standard curve is generated for a set of standard, control, and samples assayed.
Figure 1


10
1
0.1
0.01
Optical Density (OD)
10
100
1000
Activin A Concentration (pg/mL)