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Fecal Eosinophil Cationic Protein and Serum Immunoglobulin E in Relation to Infant Feeding Practices

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Fecal Eosinophil Cationic Protein and Serum Immunoglobulin E in Relation to Infant

Feeding Practices

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Guarantor: JLH

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Abstract

Background: To date, the effects of exclusive breastfeeding duration and timing of solid food introduction on allergy prevention are unclear. The aim of this study was to determine the effect of variable feeding practices on intestinal inflammation in infants using fecal eosinophil cationic protein (ECP) as a surrogate marker, and to assess whether fecal ECP is associated with serum immunoglobulin E (IgE).

Methods: Subjects (N=206) were enrolled from the Prediction of Allergies in Taiwanese Children (PATCH) birth cohort study. Stool samples were collected at 6 and 12 months for determining ECP, and blood was collected for determining total and allergen-specific IgE at 12 months. We compared these biomarkers between infants with variable exclusive breastfeeding duration and infants introduced to solid foods at various periods. The association between fecal ECP, total serum IgE and specific IgE was also analyzed.

Results: Fecal ECP were significantly higher in exclusively breastfed infants compared to formula-fed infants and infants that were not exclusively breastfed at 6 months of age ($P < 0.05$). At 12 months, infants that were introduced to solid foods at 5-6 months had the lowest fecal ECP compared with those that were introduced at earlier and later periods. There was no significant association between fecal ECP and serum IgE.

Conclusion:

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We found that breastfeeding exclusively for >6 months did not reduce serum IgE, but rather increased intestinal inflammation. Fecal ECP was not associated with total serum IgE and specific IgE and might not be a useful indicator of IgE sensitization in infancy.

Keywords:

Atopic sensitization; feeding practices; eosinophil cationic protein; IgE; infants; stool

INTRODUCTION

Because of the immaturity of the infant mucosal immune system, exposure to food antigens early in life could greatly influence the development of infant gut immunity [1]. Whether these food antigens would eventually lead to immunoglobulin E (IgE)-mediated sensitization or oral tolerance in young infants is uncertain, and seems to depend on a variety of factors [2]. Breast milk is the most important food to infants. Despite the fact that protection against infectious diseases is observed among breastfed children [3], it is uncertain if breastfeeding for longer periods is an effective strategy for preventing allergies [4-7]. In addition, the timing of solid food introduction for allergy prevention is also a matter of debate [4, 8-10]. Thus, investigations of infant feeding practices and their association with later atopic diseases are of interest to physicians.

As allergic diseases are not easy to diagnose in young infants, some well-established allergic biomarkers, such as total IgE and allergen-specific IgE, have been used for many years to promptly identify infants with atopic sensitization [11-14]. Infants with atopic sensitization have a higher risk of developing allergic diseases in early childhood [15-17].

As interactions between feeding practices and gut immunity are complicated and difficult to evaluate, using non-invasive parameters to elucidate intestinal inflammation would be helpful in clinical work. Eosinophil cationic protein (ECP) is an excellent marker for eosinophil activation in various allergic and gastrointestinal diseases [18]. Elevated

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ECP in serum and/or the presence of ECP in respiratory secretions have been shown to be of value in predicting subsequent wheezing and even asthma in infants [19-21]. Additionally, ECP as a fecal marker of intestinal inflammation can help in the diagnosis and monitoring of food hypersensitivity reactions [18, 19, 22, 23]. Currently, data regarding fecal ECP levels in healthy infants is scarce [24], particularly in relation to food practices [25].

In the present study, our first aim was to use fecal ECP as a marker for intestinal inflammation to monitor the intestinal inflammatory reactions between various feeding practices. Our second aim was to investigate the associations between risk factors, feeding practices, and IgE sensitization from birth to 12 months of age. Our third aim was to assess if fecal ECP was correlated to serum IgE and if it could be used as an indicator of atopy in clinical work.

METHODS

Study Subjects

All participates in this study were enrolled from the Prediction of Allergies in Taiwanese CHildren (PATCH) study. In March 2012, we began to recruit healthy pregnant women from the outpatient clinics and the delivery rooms of the Chang Gung Memorial Hospital, Keelung, Taiwan. Study subjects were enrolled after obtaining written informed consent. This study was approved by the Ethics Committee of Chang Gung Memory Hospital

(101-4361C).

A total of 376 infants (10 twins) were enrolled soon after birth in the birth cohort study from March 2012 to October 2013. To avoid potential confounding factors, 39 neonates (7 twins) who had a gestational age of <36 weeks, any perinatal insults, or major congenital anomalies were excluded. Eighty participants who dropped out during the 1-year follow-up period and 51 participants that did not provide stool samples were also excluded. Finally, our analytical sample included 206 infants (three sets of twins).

Questionnaires

The study participants returned for follow-up clinic visit regularly and were checked by a neonatologist at 2, 4, 6 and 12 months. The parents of the enrolled participants answered the standardized questionnaires under the guidance of well-trained research assistants at all follow-up clinic visits at 2, 4, 6, and 12 months. Detailed information about one or both parents having a physician-confirmed history of asthma, rhinoconjunctivitis, atopic eczema, urticarial, and/or food allergy, and other questions regarding the infants' demographic data, general health information, current feeding practices, timing to introduce solid foods, and clinical atopic symptoms were also collected.

Determinations of serum allergen-specific and total immunoglobulin E (IgE)

Blood samples (3-5 mL) were collected from each infant at 12 months of age. The allergen-specific IgE was determined by ImmunoCAP™ Phadiatops infant (Phadia,

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Uppsala, Sweden), and total IgE was measured by ImmunoCAP (Padua). Phadiatop infant is an in vitro test for graded determination of immunoglobulin E (IgE) antibodies specific to food and inhalant allergens (i.e. house dust mite, cat, dog, birch, timothy, ragweed, wall pellitory, egg white, cow’s milk, peanut and shrimp) that are relevant in the development of atopy in young children [14, 26]. Infants were characterized as IgE-sensitized if their ImmunoCAP™ Phadiatops levels were ≥ 0.35 PAU/L [14, 27].

Definitions used in this study

Maternal/Paternal allergy: parents who self-reported a physician confirmation that they had allergic diseases, including asthma, rhinoconjunctivitis, eczema, urticarial or food allergy, in conjunction with a response to anti-allergic treatment.

Never exclusively breastfed infants: infants that were exclusively on formula or that were partially breastfed.

Infants who were IgE-sensitized: infants who had ImmunoCAP™ Phadiatops levels of ≥ 0.35 PAU/L.

Infants who were not IgE-sensitized: infants who had ImmunoCAP™ Phadiatops levels of < 0.35 PAU/L.

Stool sample collection and processing

The infants’ stools were collected in plastic containers within 2 days before the study participants returned to the clinic for follow-up at 6 and 12 months of age. The methods of

stool sample collection and processing has been described in our previous study [28].

Briefly, our research assistants weighed each stool sample and added an extraction buffer containing citrate after stool samples collection. The stool samples were then mixed homogenously and centrifuged for 15 min at 3000 rpm and 4°C. The supernatant was collected and frozen at -80°C until it was used.

Determining the levels of fecal ECP by enzyme-linked immunosorbent assays

(ELISA)

After thawing the infant stool sample supernatants, fecal ECP concentrations (ng/mL) were determined using an eosinophil cationic protein ELISA kit (MyoBioSource, San Diego, CA, USA), according to the manufacturer's instructions. To derive ECP levels in the infant stool (g), the measured fecal ECP units were adjusted and expressed as nanogram per gram of stool (ng/g).

Statistical Analysis

The infants' demographic data were collected by questionnaires and analyzed. Numerical variables were summarized by mean $\pm$ standard deviation (SD) or frequencies and percentages. Associations between categorical variables were studied by means of the chi-square test. Analysis of variance (ANOVA) test was used to compare continuous variables with normal distribution. The differences between continuous variables with non-normal distribution were estimated with the Kruskal-Wallis test. *P*-values <0.05 were

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considered significant. Correlation coefficients between fecal ECP and serum IgE levels were determined using the Spearman rank correlation test. Statistical analysis was performed using SPSS 20.0 for Windows (Chicago, IL, USA).

RESULTS

Clinical characteristics

A total of 206 infants were enrolled. The demographic characteristics of the participants are shown in Table 1. There was a great variation in exclusive breastfeeding duration (mean: 4.7± 4.6 months) among the participants. According to the information obtained from the questionnaires, 56 (27.2%) infants were never exclusively breastfed, 64 (31.1%) infants were exclusively breastfed <6 months, and 86 (41.7%) infants were exclusively breastfed for >6 months during the first year of life. Most of the participants (81.7%) started eating solid foods at 4– 6 months (mean: 5.5± 1.4 months). Only one (0.8%) infant started eating solid foods < 4 months, and 36 (17.5%) infants started ≥ 7 months. There are very little differences in exclusive breastfeeding duration and the age at which solid food was introduced between infants with and without maternal/ paternal allergy.

The relationship between feeding practices, fecal ECP, and serum IgE levels

A total of 165 stool samples at age 6 months, and 157 stool samples at age 12 months were collected from 206 infants, respectively. Table 2 shows ECP and total serum IgE levels in relation to the various feeding practices. At 6 months, fecal ECP levels were

significantly higher for exclusively breastfed infants compared to those that were exclusively fed with formula (184.69 ± 105.87 ng/g vs. 131.42 ± 78.88 ng/g; $P = 0.012$). Additionally, the fecal ECP levels between the various exclusive breastfeeding durations (≥ 6 months, <6 months, and never) were different ($P=0.027$). Further analysis showed that infants that were exclusively breastfed for ≥ 6 months had significantly higher fecal ECP levels compared to those that were never breastfed (184.69 ± 105.87 ng/g vs. 132.03 ± 74.83 ng/g; $P=0.036$). By 12 months of age, both fecal ECP and serum IgE levels showed an upward trend in infants that were exclusively breastfed and breastfed for at least 6 months compared to the levels in infants with other feeding practices, but the differences were not statistically significant ($P > 0.05$).

For timing to introduce solid foods, the lowest fecal ECP was found in infants that were introduced to solid foods at 5- 6 months compared to infants that were introduced to solid foods earlier or later by 12 months of age ($P=0.059$). The fecal ECP at 6 months of age and serum total IgE levels at 12 months of age were not affected by the time of solid food introduction ($P > 0.05$; Table 2).

The characteristics of infants that were IgE-sensitized at 12 months

A total of 146 infants provided venous blood for allergen-specific serum IgE tests at age 12 months. Among them, 71 infants (48.6%) were defined as IgE-sensitized and 75 (51.4%) were defined as not IgE-sensitized. The characteristics of infants that were, or

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were not, IgE-sensitized are shown in Table 3. There was very little difference between both groups, including maternal factors, parents’ allergic histories, the mean duration of exclusive breastfeeding and timing of complementary food introduction, except that fewer IgE-sensitized infants had never been exclusively breastfed ($P=0.013$). The fecal ECP levels were no different between both groups at 6 and 12 months ($P > 0.05$).

Correlation between fecal eosinophil cationic protein and total serum

Immunoglobulin E levels

In assessing the relationship between fecal ECP and total serum IgE levels, only a weak, positive correlation between fecal ECP and serum IgE levels at age 12 months was observed ($r=0.124$, $P=0.061$). There was no statistically significant relationship between fecal ECP levels at 6 months and total serum IgE levels at 12 months ($r=-0.08$, $P=0.258$; Figure 1).

DISCUSSION

This study reported infants’ feeding practices in Taiwan. To the best of our knowledge, this is the largest study that used fecal ECP to monitor intestinal inflammation in infants, while also investigating the relationship between fecal ECP, total serum IgE, and allergen-specific IgE.

Breastfeeding is the preferred method of infant nutrition. In our study, only 36 infants (17.4%) were never breastfed, and 41.7% of lactating mothers chose to continue

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4 exclusive breastfeeding while solid foods had been introducing to their children after 4
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7 months of age. In general, the feeding choices, exclusive breastfeeding duration, and the
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13 regardless of one or both parents having an allergic disease.
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16 Many studies have attempted to assess the role of breastfeeding in the development
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18 of allergic disease, but the findings are inconsistent [4-7, 29]. Although expert bodies
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20 recommend that infants should be exclusively breastfed for 3-4 months to decrease the
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22 risk of allergies [4, 30], it is uncertain that prolonged breastfeeding also has protective
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24 effects, because there was a low number of infants that were exclusively breastfed for
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26 longer than 6 months in those study populations [4]. In immunological food intolerance,
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28 mast cells, eosinophils, mucosal lymphocytes, and epithelial cells are involved and
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30 related to each other in the development of mucosal inflammation [31]. Fecal ECP is an
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32 intestinal inflammatory marker that could be a promising noninvasive tool in monitoring
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34 intestinal inflammation in infants with food allergies [19, 23]. Our results show that
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36 fecal ECP levels were significantly higher in exclusively breastfed infants compared to
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38 formula-fed infants and infants that were never exclusively breastfed by the age of 6
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40 months ($P < 0.05$) (Table 3). Perhaps an explanation for increasing mucosal
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42 inflammation in prolonged exclusively breast-fed infants (≥ 6 months) was imperfect
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44 oral tolerance against all the antigenic food proteins in the breast milk. Nevertheless,
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there was no evidence showing that a longer duration of exclusive breastfeeding increased IgE sensitization at the age of 12 months (Table 3). Exclusive breastfeeding seemed not the major determining factor that influenced IgE sensitization in the first year of life.

Currently, the optimal age for the introduction of solid foods varies greatly [4, 8-10]. Several studies investigated whether early introduction of solid foods, before 4 months, or delayed introduction, after 6 months, would promote or prevent allergies [4, 9, 29, 32]. In the present study, we found that infants who were introduced to solid foods at 5-6 months had the lowest fecal ECP levels by 12 months of age, compared with infants that were introduced to solid foods earlier or later than 5-6 months. This indicates that exposure to solid food antigens during this period was not inducing an overt intestinal inflammatory response. In our study, there was no evidence showing that early or late introduction of solid foods influenced IgE sensitization in the first year of life (Table 3).

Silva AC et al. [24] reported that the mean and median fecal ECP levels were 1.93 µg/g and 1.20 µg/g, respectively, in 51 healthy Caucasian children (mean age 2.89 y). Those fecal ECP values are significantly higher than ours (median 124.24 ng/g, mean 145.20 ng/g). The difference could be due to the different methods of processing the stool samples, experimental conditions, and differences in race and age. Currently, there is no standard technique for quantification, and the fecal ECP findings vary greatly and do not follow a

normal distribution [18]; therefore, comparing the results of the few pediatric studies published to date is difficult [23, 24].

Both ECP and IgE are important mediators of allergic inflammation, and they are used to monitor allergic diseases [11,20, 21]. ECP is an intestinal inflammatory marker for studying gastrointestinal disorders that are predominantly eosinophilic [18, 23, 24, 33]. Our results showed only a weak correlation between fecal ECP and serum IgE levels at 12 months ($r=0.124$, $p=0.061$) (Figure 1). Also, there was no difference in fecal ECP levels at 6 and 12 months between infants that were or were not IgE-sensitized (Table 3). These findings suggest that fecal ECP might not be a useful indicator of IgE sensitization in infancy.

The strength of this study was its design. Detailed information regarding feeding practices and demographic characteristics of infants were obtained on a regular basis by questionnaires, and both blood and stool samples were collected prospectively. The timings of feeding practices matched the timings of sample collection. A major limitation of the study is that the number of participants was not large enough to make any definitive conclusions. Another limitation is that we did not investigate the effect of total breastfeeding duration. Additionally, the timing of specific food introduction in relation to fecal ECP levels was not evaluated.

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In summary, we found that exclusive breastfeeding for longer than 6 months did not seem to reduce serum IgE levels, but instead showed a tendency to increase intestinal inflammation. Additionally, infants starting solids food during the fifth and sixth month showed the mildest intestinal inflammation by 12 months of age. As fecal ECP was not significantly associated with total serum IgE and allergen-specific IgE levels, we suggest that fecal ECP could be considered to be a specific intestinal inflammatory marker, but not an indicator of atopy in infancy.

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Table 1. Demographic characteristics of infants (N=206)

Characteristics	Infants (N=206)
Sex of infants, male (%)	105 (51.0)
Gestational age (week)	38.4±1.2
Cesarean section, n (%)	72 (35.9)
Birth body weight (kg)	3.0 ± 0.5
Maternal age (y)	31.5 ± 6.8
Maternal education: College or above, n (%)	148 (71.8)
Maternal allergy, n (%)	89 (43.2)
Paternal allergy, n (%)	94 (45.6)
Exclusive breastfeeding duration, month	4.7± 4.6
Age at introduction of solid foods, month	5.5± 1.4

Table 2. Fecal eosinophil cationic protein and total serum immunoglobulin E levels in relation to feeding practices.

Feeding practices	Fecal ECP at 6 months		Fecal ECP at 12 months		Total serum IgE at 12 months	
	n	Mean± SD	n	Mean± SD	n	Mean± SD
		(ng/g)		(ng/g)		(KU/L)
Exclusive feeding practices since birth						
Human milk	64	184.69±105.87	27	182.11±178.57	26	119.63 ±286.71
Infant formula	36	131.42± 78.88	33	141.32± 117.43	30	40.45± 34.31
<i>P</i> -value		0.012*		0.448		0.137
Exclusive breastfeeding duration						
≥ 6 months	64	184.69±105.87	70	149.37±187.64	62	91.75±98.67
< 6 months	53	141.20±87.04	51	139.35±113.23	49	66.32±91.15
Never	48	132.03±74.83	35	126.00±67.48	34	39.54± 33.05
<i>P</i> -value		0.027*		0.708		0.242
Age at introduction of complementary foods						
≥ 7 months	29	165.74±106.13	24	180.26±111.21	26	73.87±153.57

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5-6 months	88	147.15± 85.55	85	114.07±63.84	75	65.86±75.50
≤ 4 months	48	145.32±105.40	48	161.44±223.52	45	55.21±90.80
<i>P</i> -value		0.640		0.059		0.734

**Statistically significant (*P*<0.05)

Abbreviations: ECP, eosinophil cationic protein; IgE, immunoglobulin E

Table 3. Description of the characteristics of infants that were or were not IgE-sensitized at 12 months

Characteristics	Infants who were IgE-sensitized (N=71)	Infants who were not IgE-sensitized (N=75)	<i>P</i> -value
Maternal factors			
Maternal age (y)	30.8 ±4.7	32.3 ±10.5	0.263
Maternal education level			0.837
High school or below, n (%)	20(28.2)	23(30.7)	
College or above, n (%)	51(71.8)	52(69.3)	
Family history of allergy			
Maternal allergy, n (%)	31(43.7)	33 (44.0)	0.550
Paternal allergy, n (%)	32(45.1)	31(41.3)	0.386
Neonatal factors			
Gestational age (week)	38.6 ±1.3	38.5 ±1.1	0.602
Cesarean section, n (%)	27 (38.0)	25(33.3)	0.606
Birth body weight (kg)	3.1±0.5	3.0±0.6	0.635

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Environmental tobacco	49 (69.0)	44 (58.7)	0.229
Smoking after birth, n (%)			
Household pets at 12 months, n (%)	24(33.8)	20(26.7)	0.372
Exclusive breastfeeding duration, month	5.5 ± 4.5	4.2 ± 4.7	0.119
≥ 6 months, n (%)	34 (47.9)	28(37.3)	0.242
<6 months, n (%)	26 (36.6)	22 (29.3)	0.382
Never, n (%)	11(15.5)	25(33.3)	0.013*
Age at introduction of solid foods, month	5.6 ± 1.5	5.7 ±1.6	0.679
≥ 7 months old, n (%)	10 (14.1)	16(21.3)	0.285
5-6 months old, n (%)	41(57.7)	34(45.3)	0.141
≤ 4 months old, n (%)	20(28.2)	25(33.3)	0.591
Fecal ECP levels, Mean± SD (ng/g)			

at 6 months old	160.37±98.25	147.99±88.16	0.521
at 12 months old	140.17±104.28	125.20±93.08	0.437

Infants that were IgE-sensitized were those infants that had ImmunoCAP™ Phadiatops

levels of ≥ 0.35 PAU/L.

Infants that were not IgE-sensitized were those infants that had ImmunoCAP™

Phadiatops levels of < 0.35 PAU/L.

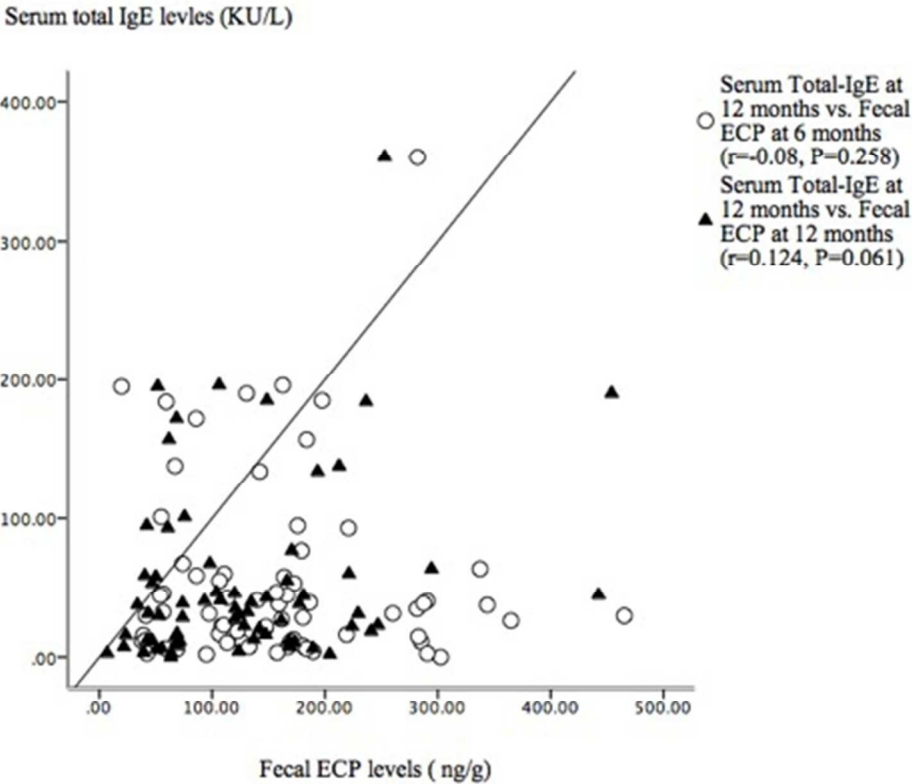


Figure 1. Scatterplot for serum total IgE and fecal ECP levels at 6 and 12-month
165x153mm (72 x 72 DPI)