Diagnostic Accuracy of Immunological Markers for Detection of Allergens by Poly Check Techniques among Patients with Bronchial Asthma in Basrah, Southren of Iraq

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ABSTRACT

This study aimed to estimate specific IgE by poly check technique against various allergens. In the present study 56 environmental allergens (food, fungal, agricultural, and aeroallergens) were tested against specific IgE. This study found a very high significant allergen – specific IgE reactions with a high degrees mediated a various forms of hypersensitivity is sever in 4 and 5 stage of age groups to males asthma patients, in allergens except Cat Ep. While in females recorded severe hypersensitivity in 2, 3 and 4 stage of age groups, in allergens except cat. Ep., Plantain, hazel pollen. And Alder pollen.

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Introduction

Asthma is a complex respiratory disease in which genetic predisposition, environmental and immunological influences interfere with each other. It is considered one of the most prevalent chronic diseases, affecting approximately 300 million individuals and causing an estimated 250,000 deaths each year. In addition, it is projected that by 2025, the global asthma burden will rise by 100 million people due to a growing Westernized lifestyle and urbanization in developing countries. The 'hygiene theory' was originally attributed to an increase in the prevalence of allergic diseases, including asthma, indicating that decreased exposure to microbes during the first years of life plays a role in the development of allergic diseases. While this theory is generally accepted, studies have shown that the increased incidence of asthma, rhinitis, or Neurodermitis does not completely account for decreased microbial exposure [1-6]. Asthma is a widespread illness globally and affects individuals of all ages, This condition usually occurs in infancy and is characterized by variable symptoms of wheeze, dyspnea, and chest tightness caused by air flow obstruction (fully reversible) [7,8].

Food allergy

An immunological reaction that occurs from food ingestion, inhalation or atopic touch. IgE antibodies or other immune cells, such as T cells, may mediate immunological reactions. Any staff specifically classify food allergies these immunological responses are mediated by immunoglobulin E (IgE) antibodies, whereas some use the wider concept of immunological response, which also includes mediated T-cell responses. Enzymes, enzyme inhibitors, structural proteins or binding proteins with diverse biological functions can be allergenic proteins in foods [9-11]. Food allergy pathogenesis starts with a process of sensitization during which the body identifies one or more proteins as a foreign invader in a specific food source and begins to mount an immune-defensive response. Subsequent ingestion of the offending food may result in an allergic reaction that may occur in either of two ways, i.e., immediate or delayed response.

Drug allergy

One form of unpredictable ADR involves a variety of hypersensitivity reactions mediated by immunology with various pathways and clinical presentations [12]. It accounts for around 5-10% of all ADRs [13]. Another form of unpredictable ADR is pseudoallergic reactions (also known as non-allergic or non-immune-mediated reactions). These reactions are mostly indistinguishable from true allergic reactions mediated by immunology, yet lack immunological precision. Not only does drug allergy affect the quality of life of the patient, but it can also lead to delayed treatment, the use of suboptimal alternate drugs, needless inquiries, and even death. In addition, considering the various signs and clinical presentations associated with the disorder, the recognition of drug allergy is difficult. Therefore, if a drug-induced allergic reaction is suspected, it is advised to consult an allergist skilled in drug allergy detection, diagnosis and treatment. This article will include an overview of drug allergy mechanisms and risk factors, as well as well as diagnostic and effective treatment methods for some of the most prevalent medications.

Materials and methods
Samples

A total of (312) patients (149 males and 163 females) of various age groups were included in this Case –control study. The patient

was examined, and diagnosed as asthma under supervision of the Physician. The study was carried out during a period from July 2018 to January 2020.

The grouping of patient
Male& Female patients were divided into five groups according to (Falk, 1993; Herd,et al.,1996 ; Nishioka,1996 ; charman&Williams,2002)
Group 1: 1- 11 years
Group 2: 12 – 2o years
Group 3: 21- 3o years
Group 4: 31 – 4o years
Group 5: above 4o years

Control group
A total of (204) healthy individual (81 males and 123 females) without any features of asthma or any allergies to be compared with asthmatic patient in genetic and immunological studies.

Statistical analysis
Statistical analysis is done by using statistical package for social sciences(SPSS) software version 11, the chi square test, univariate and multivariate logistic regression analysis, the ANoVA analysis were applied for correlation between each study parameter, and the difference between two proportion by T- tests were used to assess the significance of difference between groups, P-Value less than 0.05 was considered as Statistically significance(S).P-value < 0.01 as highly significant(HS).and P-value >0.001 as extremely significant(ES).

Polycheck Test
1- **Equipment:** Personal Computer, printer, flatbed scanner
2-**Biocheck Imaging Software (BIS)**: For patient-oriented analysis, calculation and report.
3-**Lab Equipment**: Rocking shaker (30 rpm), adjustable pipettes for 200 up to 1000μl; Multipette (1ml) to dispense the wash solution
4-**Demineralized water**: one-liter bottle for preparation of wash solution

Test performance
A-To start the assay: all component was tested at room temperature and be mixed well.
B- Reagents lots provided with the actual kit was used only.
C- Powder wash buffer has to be diluted with demineralized water at least 30 minutes prior to use avoid foaming.
D- The membranes of the test cassettes shouldn't dry during the assay.
E- All incubation steps are performed at room temperature (18-24 c) and with constant shaking.
F- A flatbed scanner with CCD sensor and scanning resolution of 600 DPI was used for interpretation of the test result.
G- Performance was tested by automated system additional information sheets are available.
1- A sufficient number of polycheck allergy cassette was prepared and mark them-only on the long side of the cassette.
2- The cassettes was moisture with 1 ml wash buffers, by tapping upside-down on absorbent paper.
3- Overlay allergy cassettes with 250 micro letter of polycheck start solution (blue cap)and incubate for 60 seconds (always pipette into the gap).Tap the cassettes carefully upside-down on absorbent paper.
4- A 200 micro letters of the respective patients serum was add into the cassette and incubate for 60 minutes on a shaker place

the MTP-holder on the middle of shaker.
5- A 3 times was decanted and washed with 1ml of polycheck wash buffer. Tap the cassettes carefully upside-down on absorbent paper.
6- A 250 micro letters was add wash buffer and incubate for 5 minutes on a shaker.
7- Repeat step 5 .Decant and tap the cassettes carefully.
8- A 250 micro letter of polycheck anti-IgE antibody was pipetted and incubated for 45 minutes on a shaker. Decant and wash 3 times with 1 ml wash buffer. Tap the cassettes carefully on absorbent paper.
9- A 250 micro letters polycheck enzyme –labeled anti-ligand was add and incubated for 20 minutes on shaker .decant and wash as described in 7.Tap the cassettes carefully on absorbent paper.
10- A 250 micro letter of polycheck substrate solution was add. And incubated for 20 minutes in the dark .decant and wash as described in 7.
11- Air dray the membrane and evaluate the polycheck allergy cassettes using scanner and the
BioCheck imaging software.

Table 1: Specific IgE Standard (Polycheck)

Class	Conc. IgE(KU/I)	Explantation
0	<0.35	No specific antibody detection
1	0.35 - <0.7	Very weak antibody conc.
2	0.7 - <3.5	Weak antibody conc.
3	3.5 - < 17.5	Clear antibody conc.
4	17.5 - < 50	Strong antibody conc.
5	50 - < 100	Very strong antibody conc.
6	>= 100	Extremely high antibody conc.

KU/I kilo unit per letter

Specific IgE (Poly check technique)
To study specific IgE concentration against (56) studied allergens, it is important to clear that these allergens (in number and specification) were studied at first time in the similar immunological studies for asthmatic patient. Illustrate in Fig. 3-7 to Fig. 3-118 also the following standard concentration used to classify the type of allergy for each patients as mentioned in Chapter 2 material and methods

Table 2: Poly Check Standard Concentration

Class	IgE concentration	Explain
0	<0.35 KU/l	No Ab
1	0.35- < 0.7 KU/l	Very week
2	0.7 - < 3.5 KU/l	Week Ab
3	3.5 - < 17.5 KU/l	Clear Ab
4	17.5 - < 50 KU/l	Strong Ab
5	50 - < 100 KU/l	Very strong Ab
6	KU/l 100≤	Extremely high Ab

Results
Figures: (1-55) illustrated the types of sensitivity of Asthma Male patients according to allergens- specific IgE reaction measured by poly check technique. From 62 environmental (food, fungal, agricultural, chemical and aero-allergens), it has been recognized that allergens which induced highest degree of reactions (highest type of hypersensitivity)
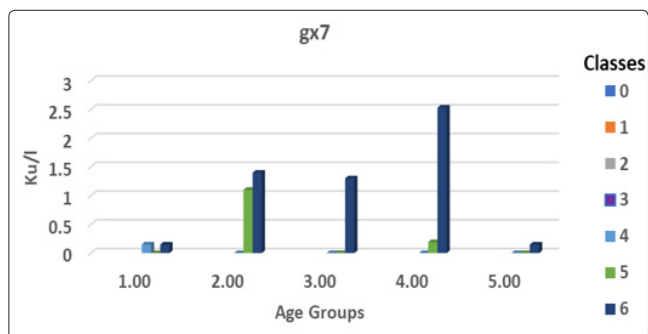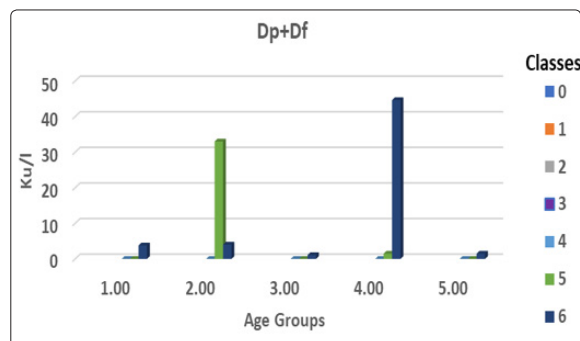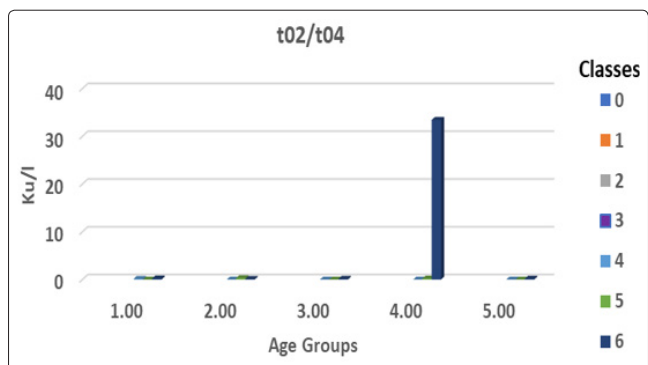
Figure 1: 6grass mix

Figure 2: Alder/Hazel pollen
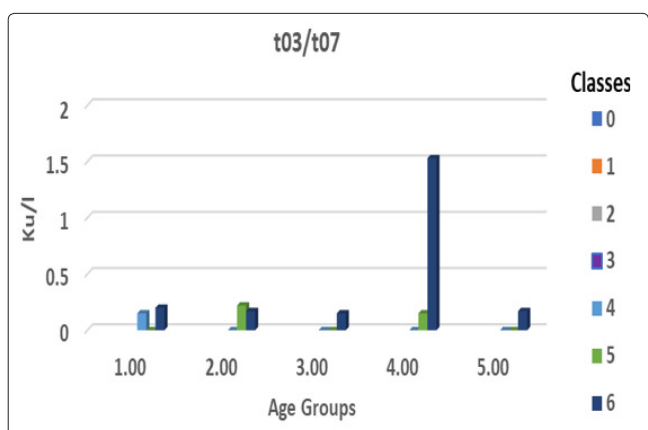
Figure 3: Birch/white Oak pollen
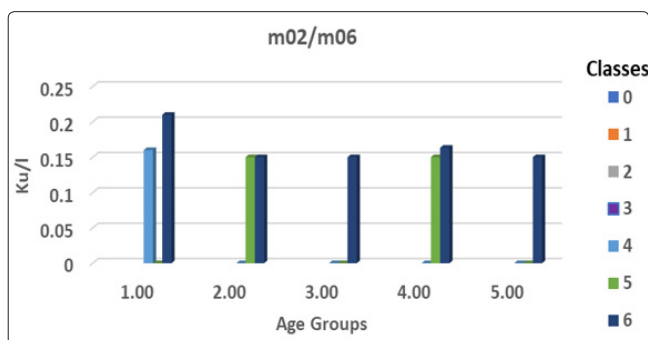
Figure 4: Cladosporium herb. /Alternaria alterna

Figure 5: D.pteronyssinus+farinae

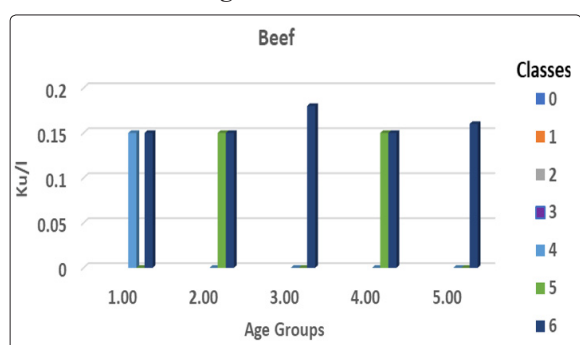**Figure 6:** Bakers yeast

Figure 7: Wheat flour
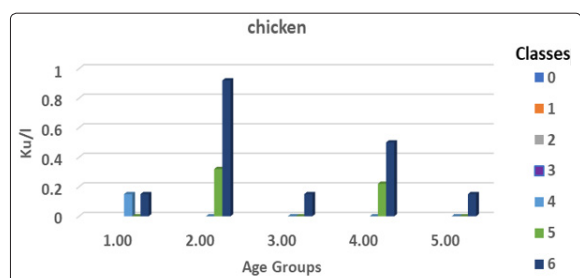
Figure 8: Chicken

Figure 9: Beef meat
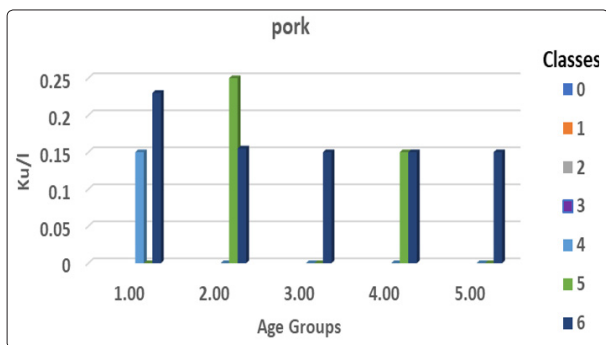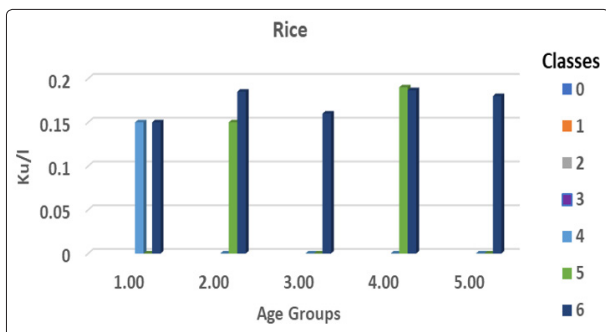
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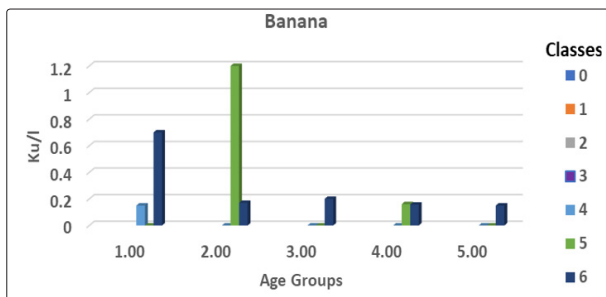
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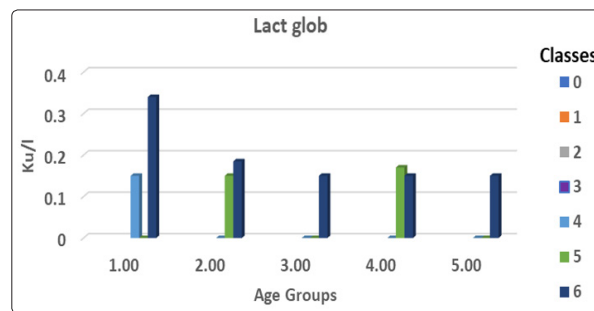
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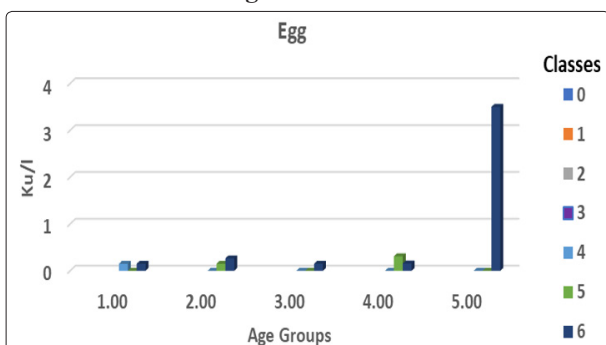
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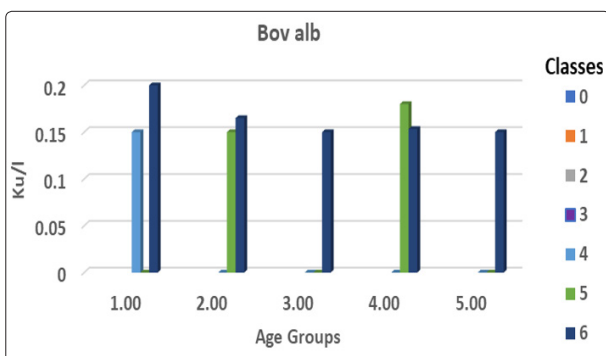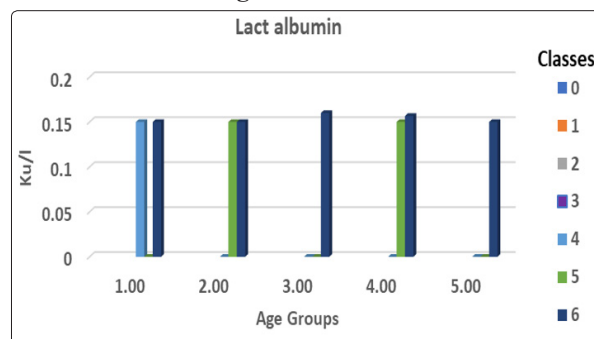

Figure 14: Bov alb

Figure 15: Bov. Alb

Figure 16: Lacto globulin
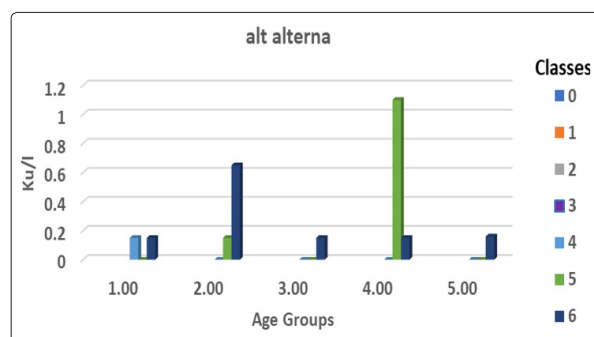
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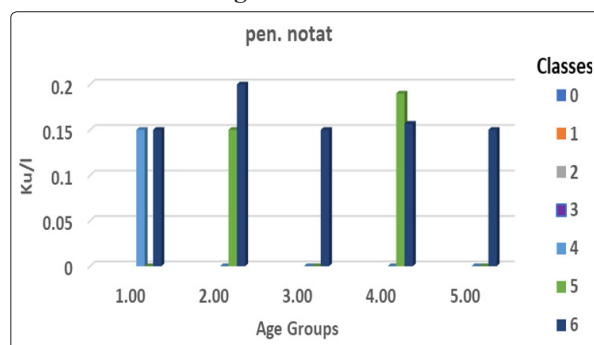
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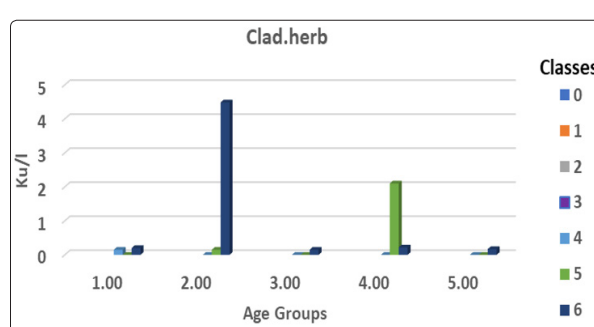
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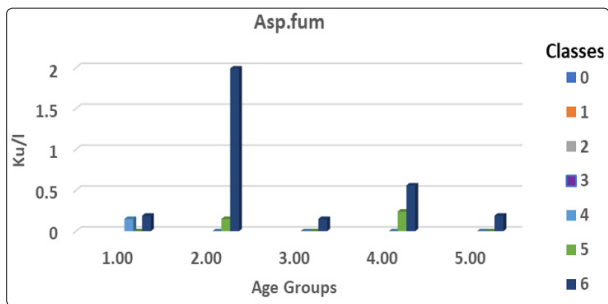
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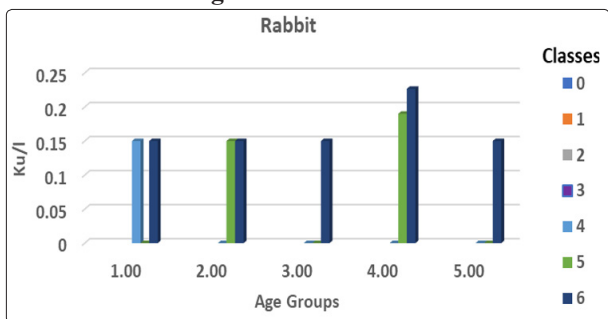
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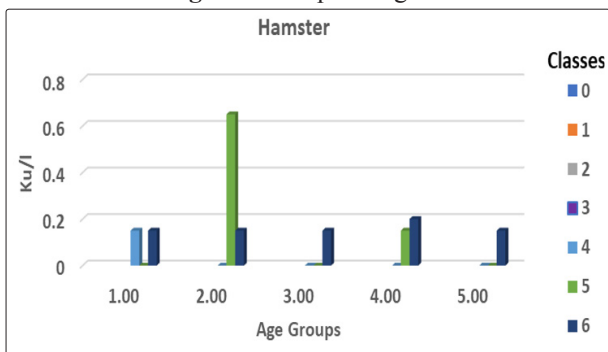
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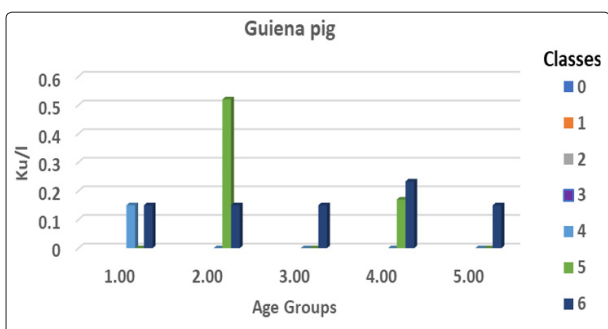
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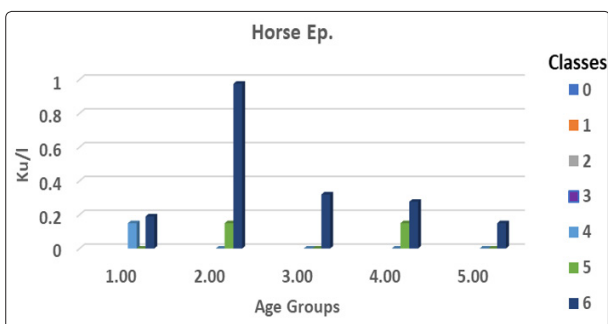
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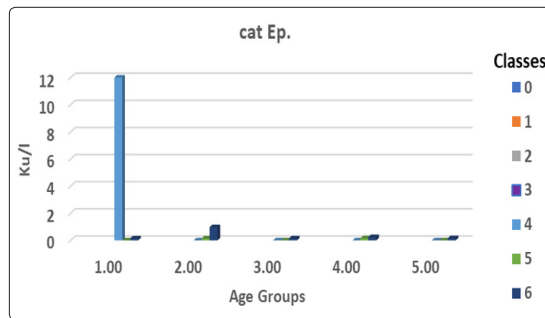
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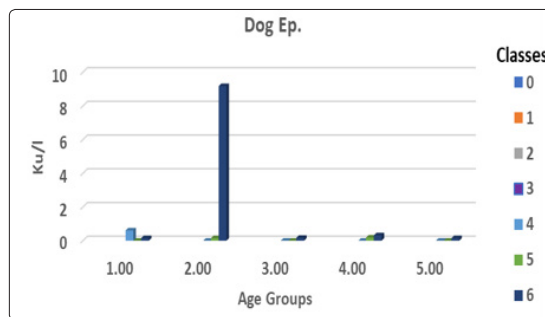
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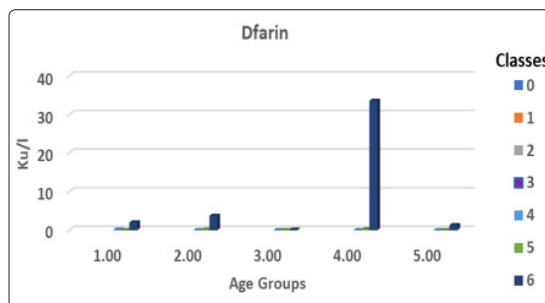
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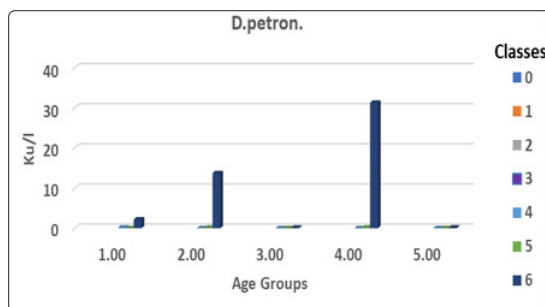
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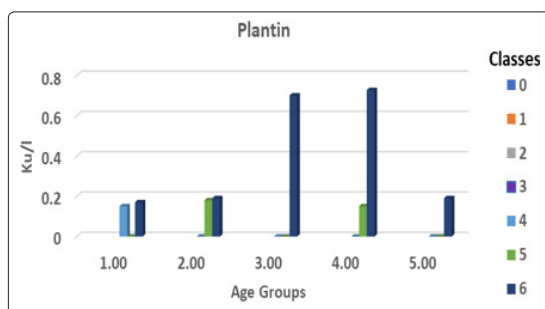
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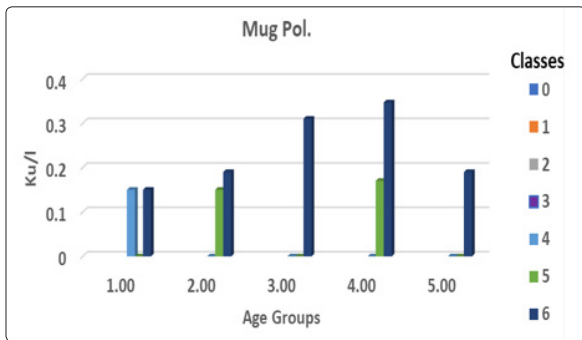
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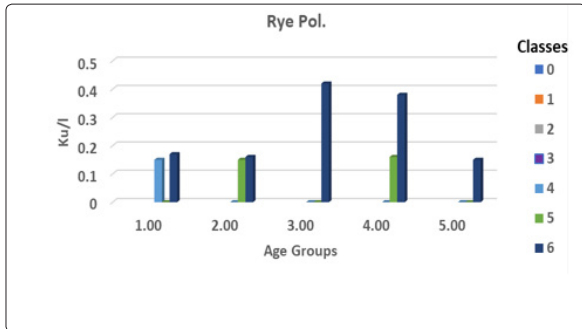
Figure 31: Mug.pol

Figure 32: Rye pol.

Figure 33: Timothy pol

Figure 34: Oak pollen

Figure 35: Hazel pol.

Figure 36: Alder pol.

Figure 37: Birch pol.

Figure 38: Rye flour
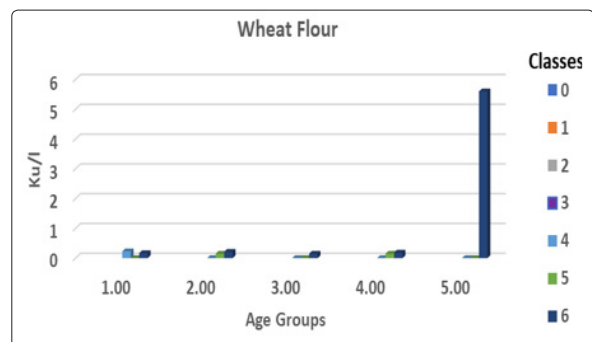
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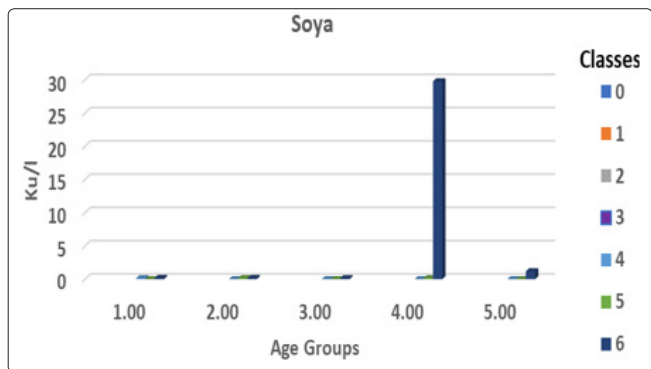
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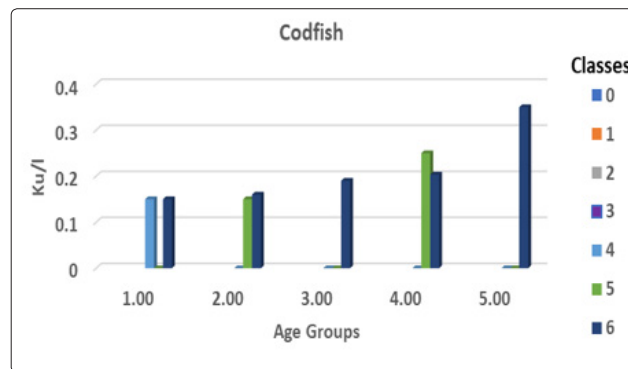
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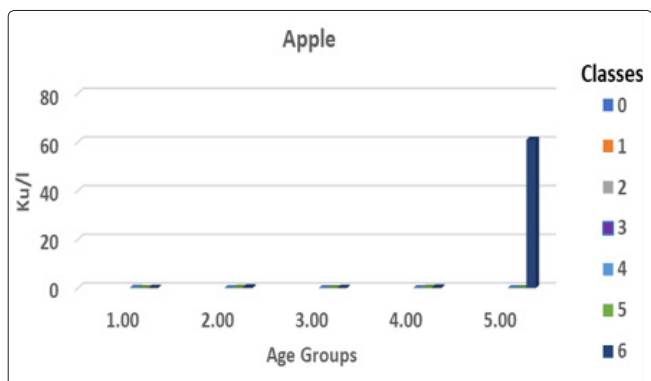
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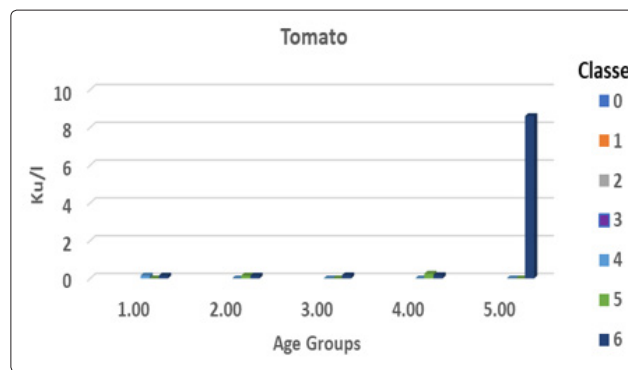
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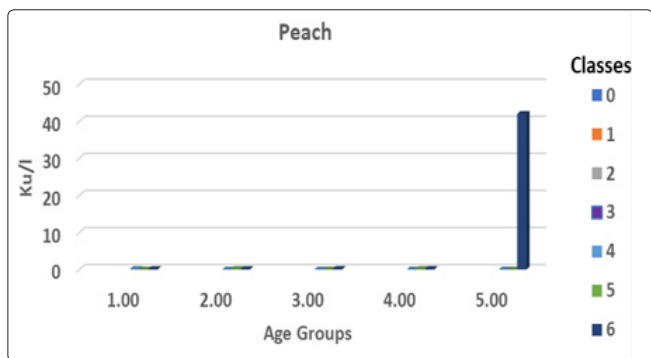
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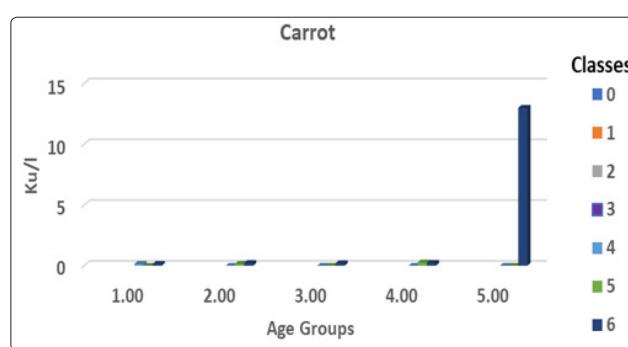
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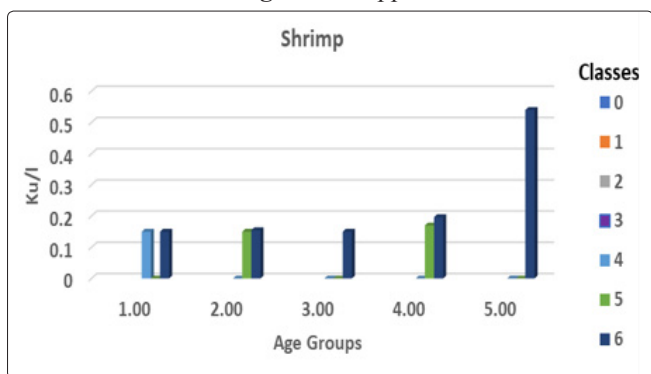
Figure: 43: Peach
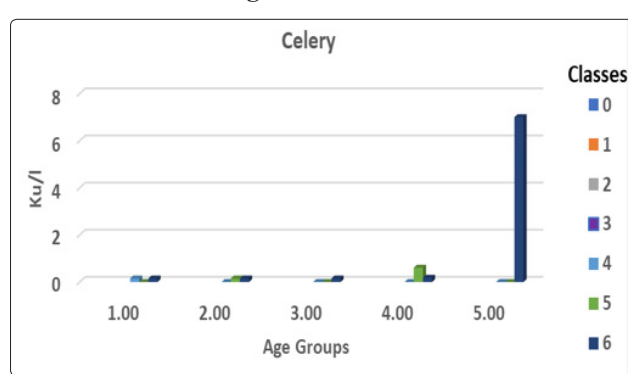
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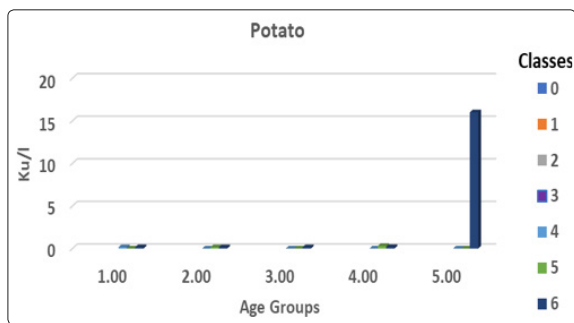
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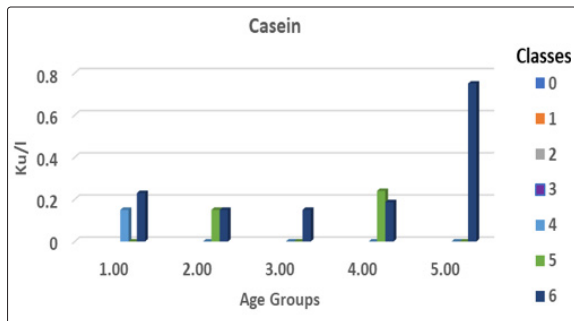
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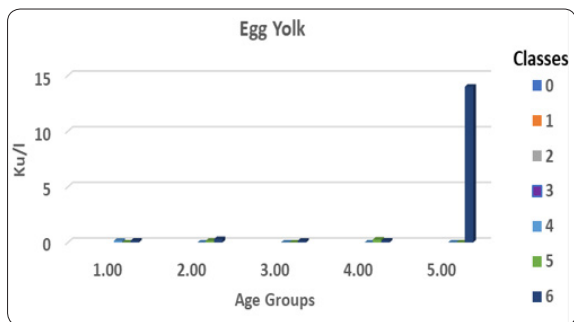
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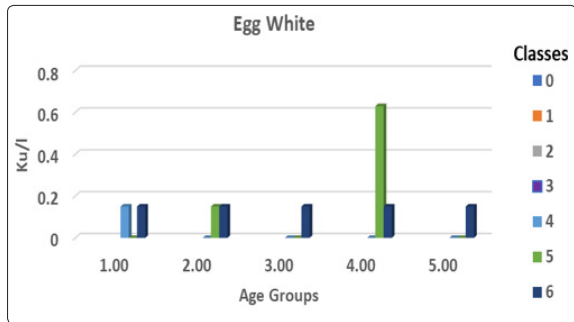
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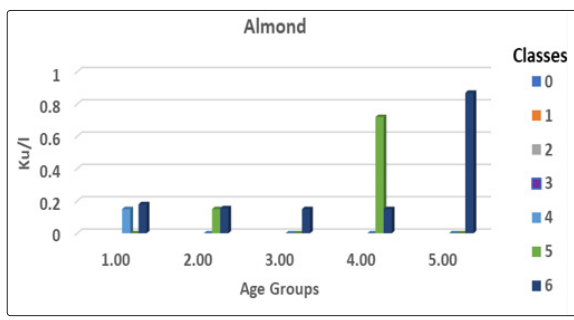
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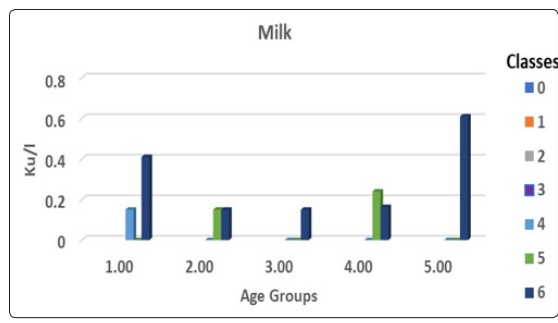
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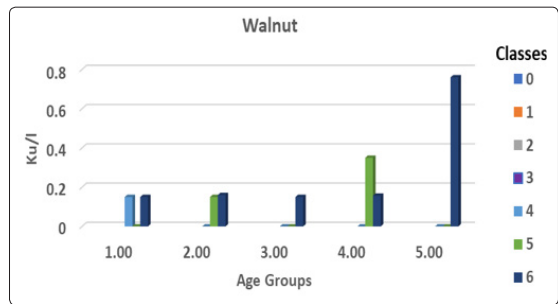
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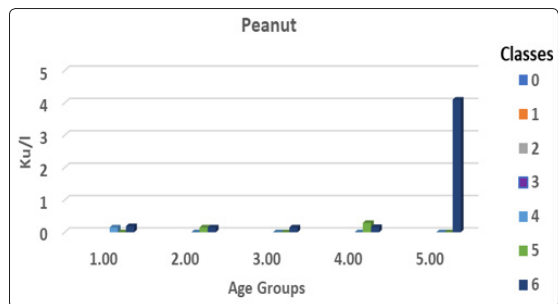
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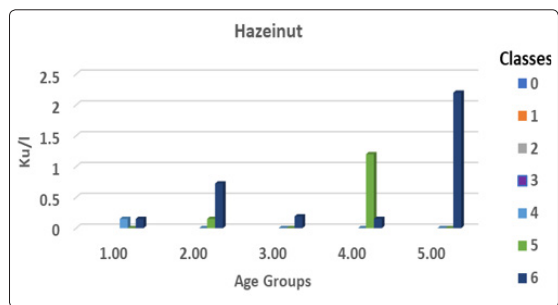
Figure 56: Peanut

Figures (57-112) illustrated the types of sensitivity of Asthma Female patients according to allergens- specific IgE reaction measured by polycheck technique. From 56 environmental (food, fungal, agricultural, chemical and aero-allergens), it has been recognized that allergens which
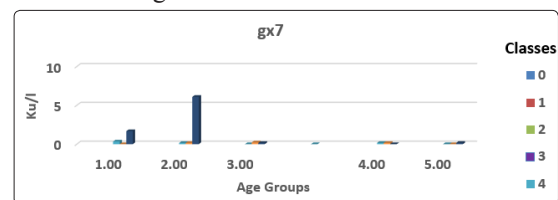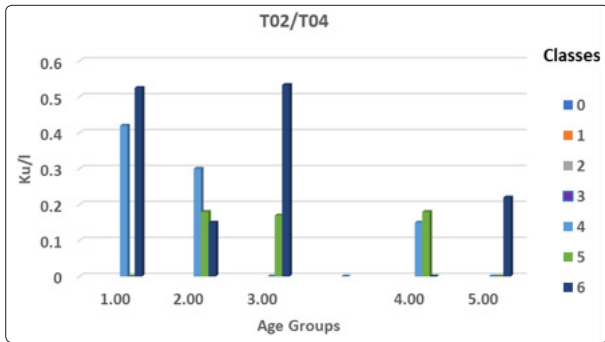

Figure 57: 6grass mix
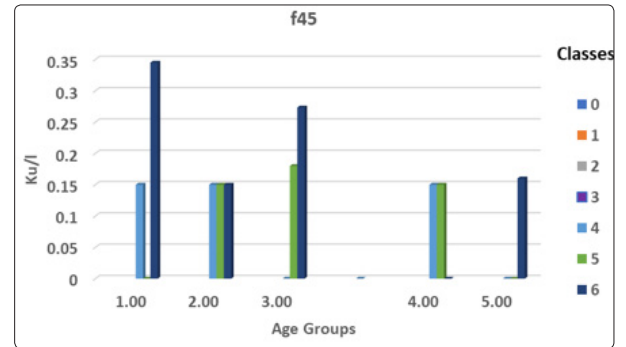
Figure 58: Alder/Hazel pollen
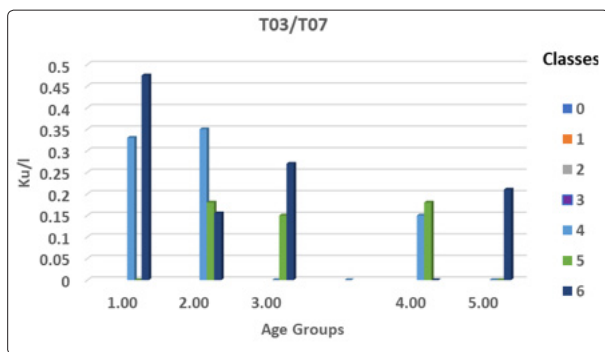
Figure 59: Birch/white Oak pollen
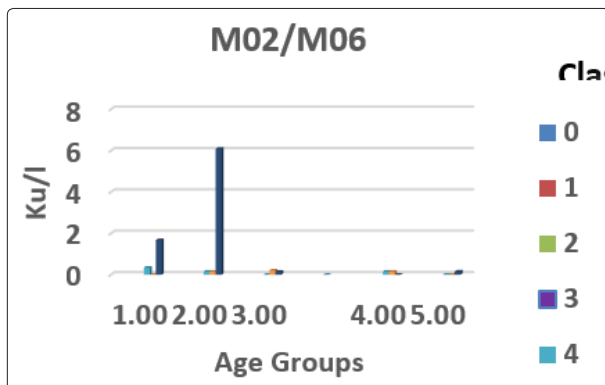
Figure 60: D.pteronyssinus+farinae
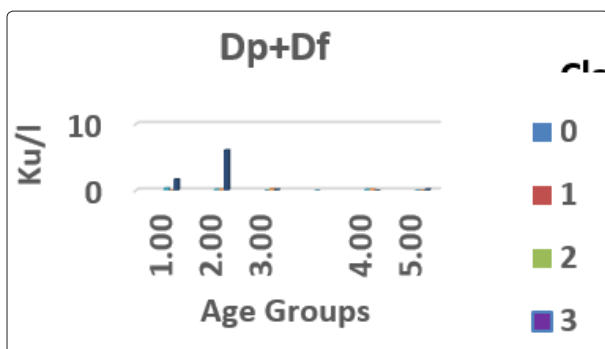
Figure 61: D.pteronyssinus+farina

Figure 62: Bakers yeast

Figure 63: Wheat flour

Figure 64: Chicken
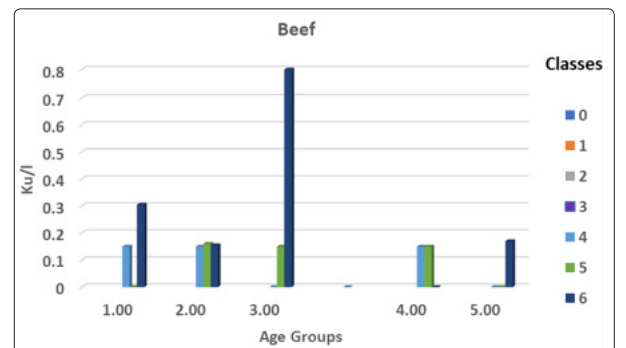
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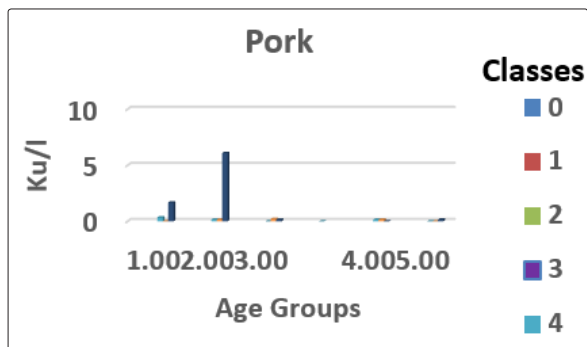
Figure 66: Pork meat

Figure 67: Banana

Figure 68: Rice

Figure 69: Egg White +yolk

Figure 70: Bovin alb.

Figure 71: Lacto glob.

Figure 72: Lacto alb.
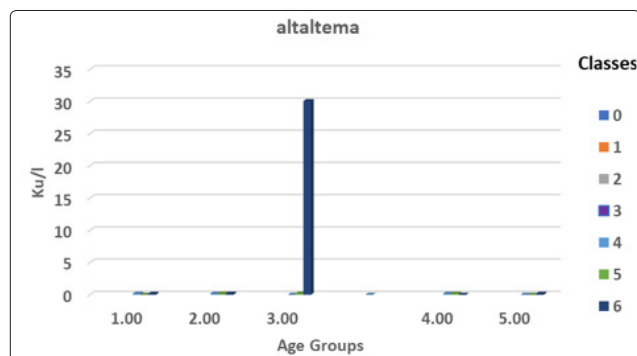
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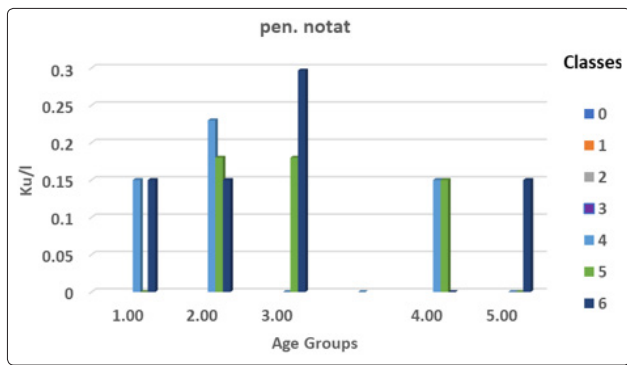
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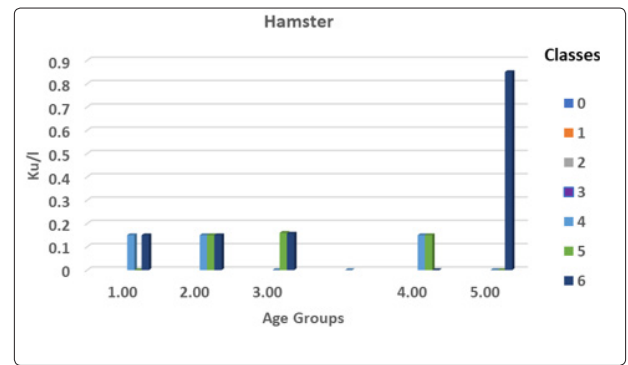
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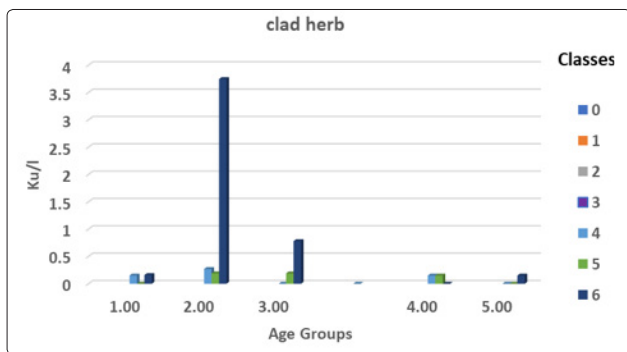
Figure 75: Clado.herb.
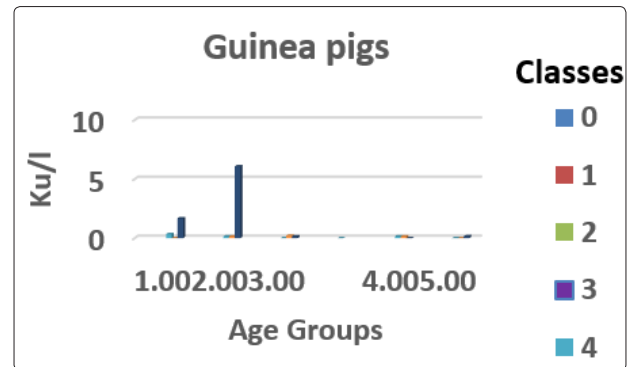
Figure 79: Guinea pigs
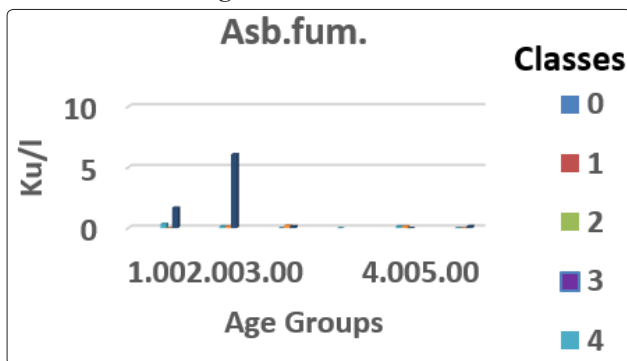
Figure 76: Asp. Fum.
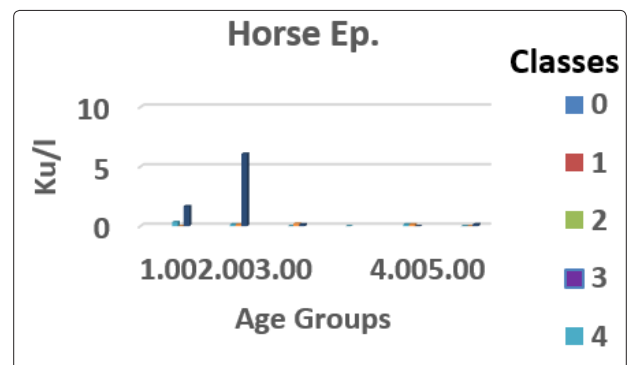
Figure 80: Horse Ep.
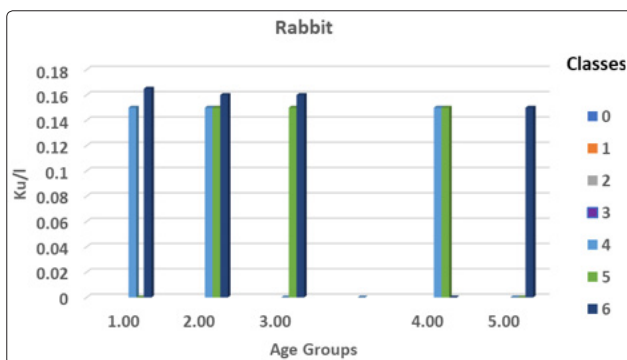
Figure 77: Rabbit
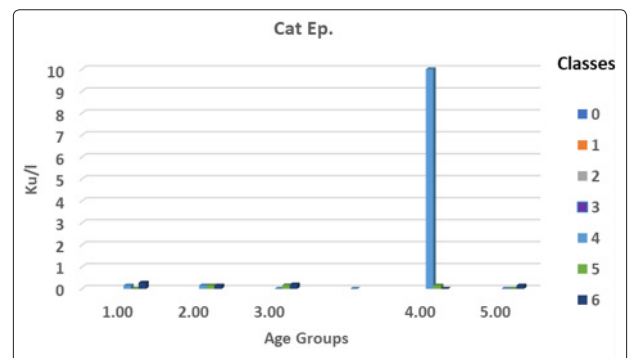
Figure 81: Cat Ep.
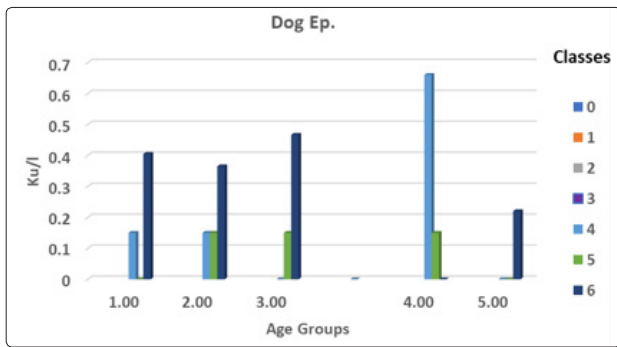
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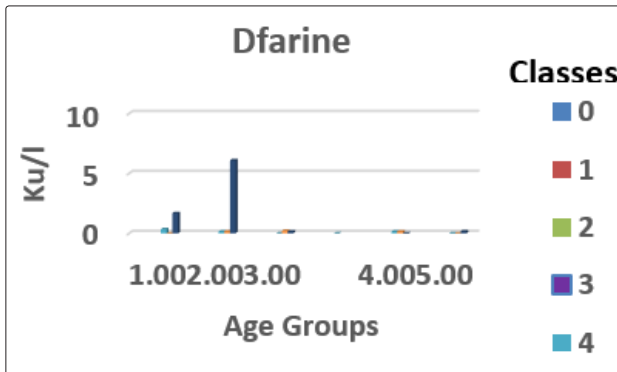
Figure 83: D. farine
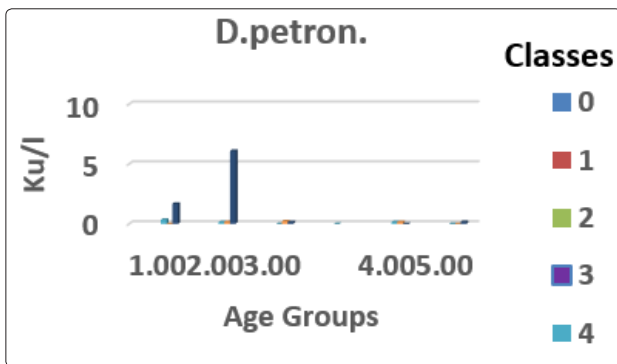
Figure 84: D.petron

Figure 85: Plantin

Figure 86: Mug. Pol.

Figure 87: Rye pol.

Figure 88: Timothy pol.
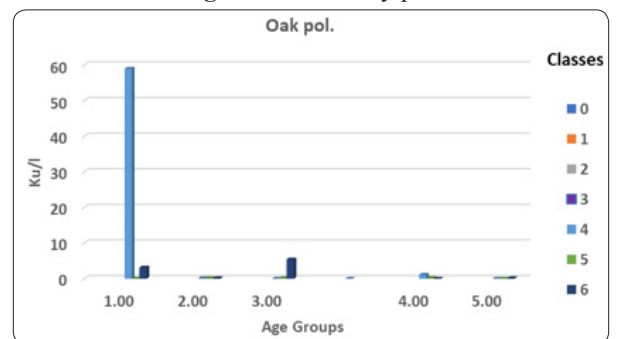
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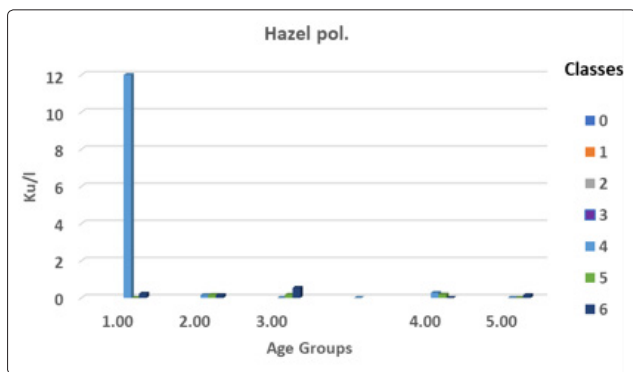
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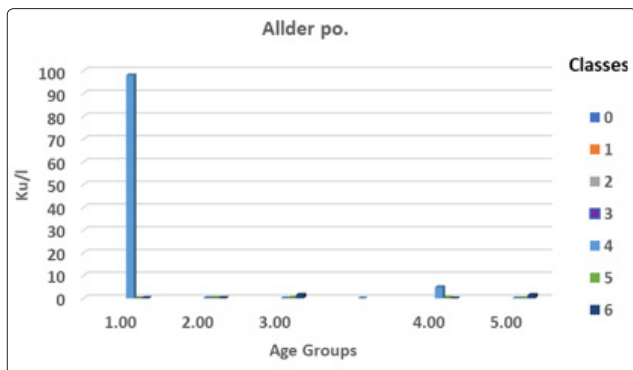
Figure 91: Alder pol.
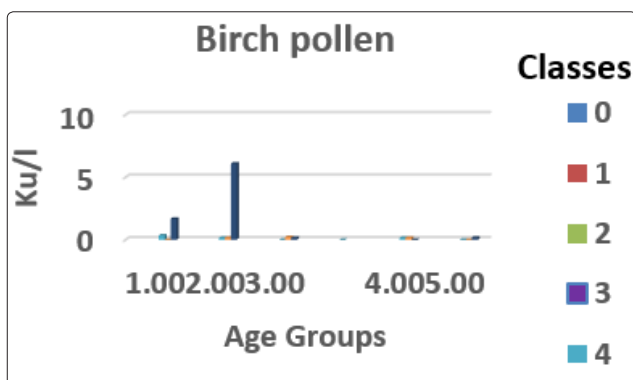
Figure 92: Birch pol.
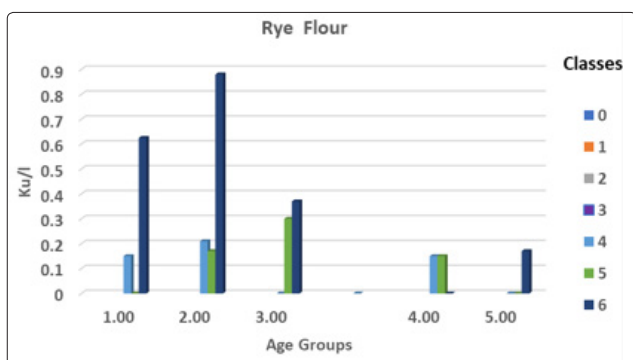
Figure 93: Rye flour
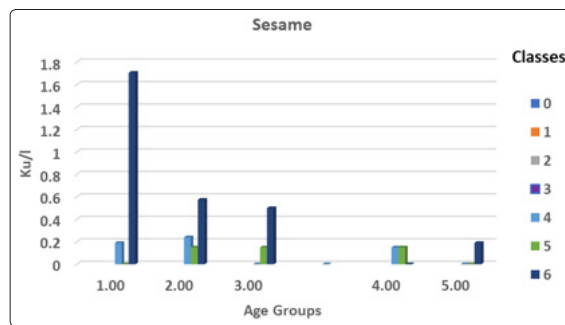
Figure 94: Sesame
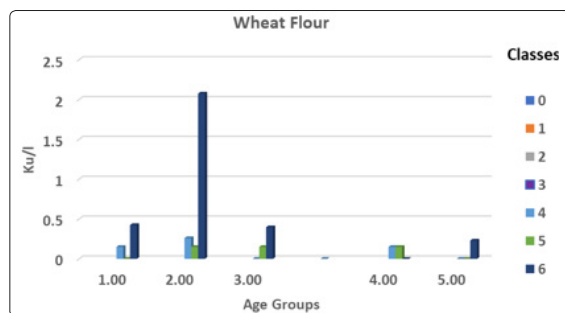
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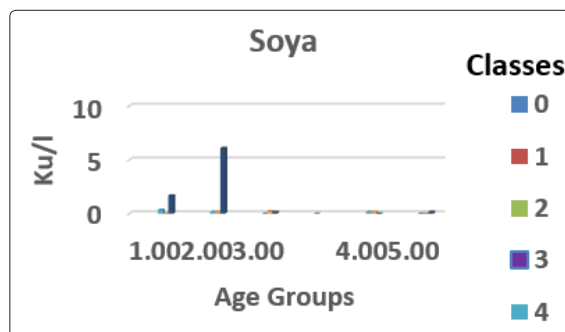
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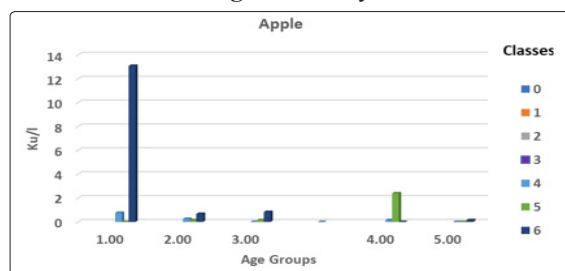
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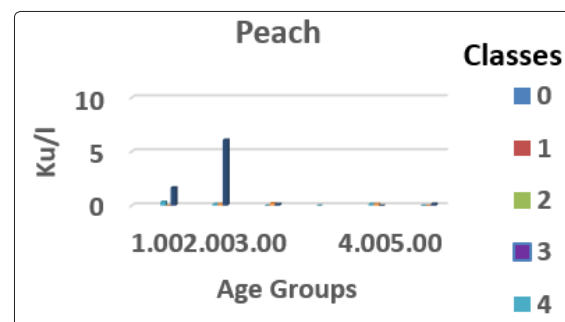
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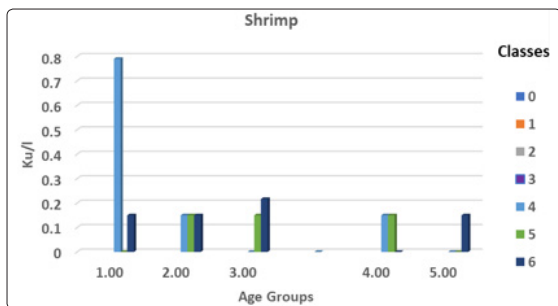
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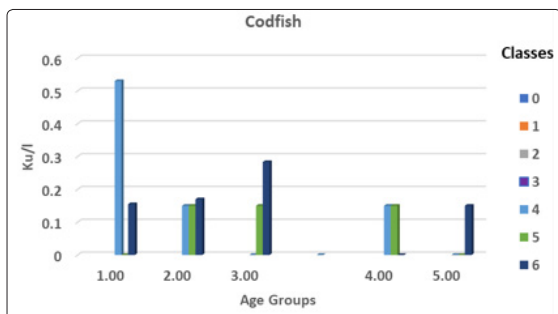
Figure 100: Codfish
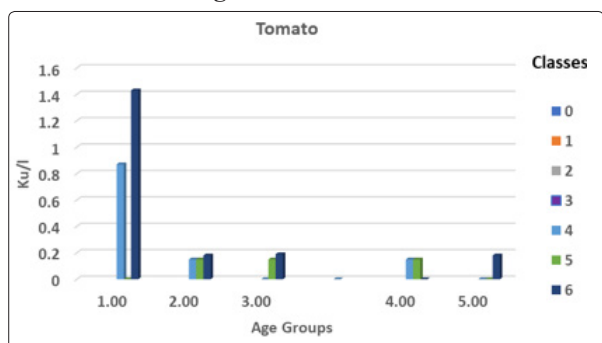
Figure 101: Tomato

Figure 102: Carrot

Figure 103: Celery

Figure 104: Potato

Figure 105: Casein

Figure 106: Egg yolk

Figure 107: Egg white
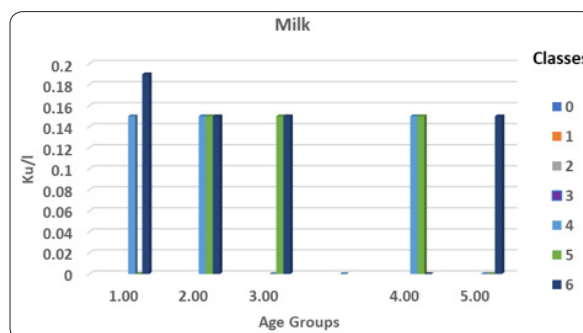
Figure 108: Milk
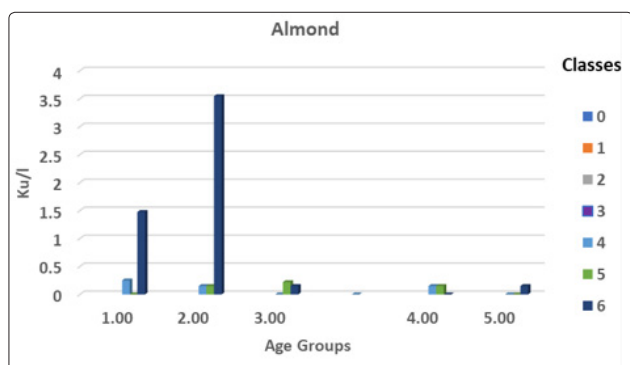
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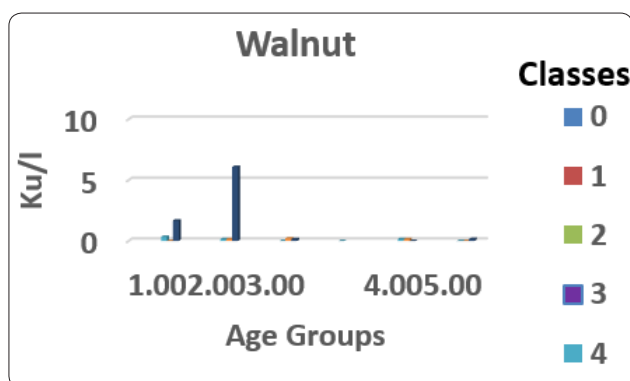
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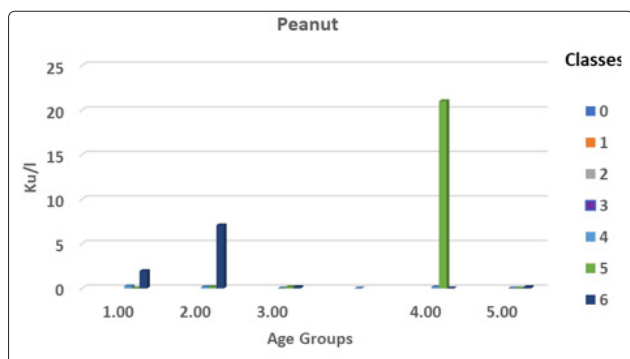
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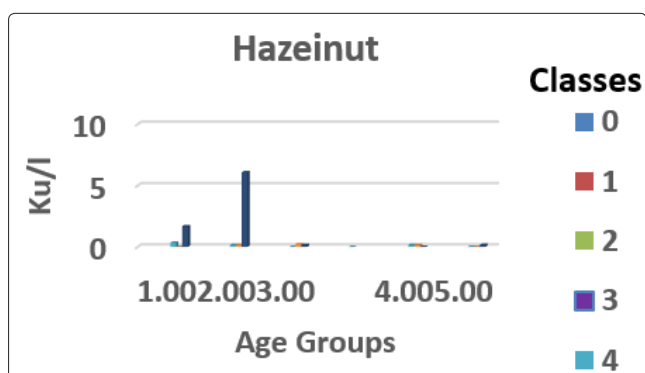
Figure 112: Hazeinut

Discussion

56 environmental allergens (food, fungal, agricultural, and aeroallergens) have been checked against particular IgE in the present research. This study found a very highly important allergen-specific high-grade IgE reactions induced by a number of forms of hypersensitivity in male asthma patients in 3 and 4 age groups in allergens except Cat Ep. While in females recorded severe hypersensitivity in 2,3 and 4 stage of age groups, in allergens except cat. Ep., Plantain, hazel pol. And Alder pol. Our result interested in allergen – specific IgE reactions which were absolutely compatible and agreed with the results of previous studies that have been carried out about atopic disease in respect of bronchial asthma as follows: Allergies to food can lead to life-threatening reactions and decrease the quality of life. Most reactions are triggered by a minority of these foods, and popular food allergens differ across geographic regions. [14] Serum IgE levels are elevated in high percent of AD children, and positive skin tests and RASTS to many allergens to diet and environment exist in high percentage of children with AD Suratannon. Food allergic reactions cause increased skin symptoms in about (33-40%) children with moderate to severe disease [15-18]. Reported that out of 74 Swiss children have atopic dermatitis, 34% of them had hypersensitivity. Patient might have both Food allergy and asthma, nearly in one-third of children with food allergy having asthma. This leads to risk of having a fatal reaction from food allergen exposure. For this reason patients should be carefully instructed on allergen. [14]. Many types of food allergens have been studied that were found to cause or related with atopic dermatitis in various ages such as:

1. Proteins (David, etal., 1984 ; Spergel, etal., 1998, and Schade, et al., 2000) . Peanut (Lester, M. R. (2014).
2. Soybean (James JM, Crespo JF 2007, Komata T et al. 2009).
3. Shellfish (Stephen H et al. 2009 and Niggemann et al. 2000).
4. Wheat (Mansueto P et al. 2015, Komata T et al. 2009).
5. Milk (Waser M et al. 2007 and Schade 2000).
6. Egg (Vazquez-Ortiz et al. 2014 and Li et al. 2001).
   And others (Peanut, Soy, Vegetables, tree nut, Seafood, and others) (Niggemann, etal ., 2000 ; Worm, etal., 2000 and Grunther & Sampson, 2002).

Changes in the weather lead to allergen production, which have impact on severity of allergic reactions (Nielsen et al., 2002; Singer et al., 2005).

Recent studies suggested that the quantity of specific IgE for many allergens measured by serum assays of patients with atopic dermatitis. IgE to dust mite allergens in patients with AD were in general at least 20- fold higher than in sera from patients with asthma. Some of these results were found in our study that evidenced the strongly relation between various aeroallergens and the concentration of specific IgE. Modern studies linked type one and type four hypersensitivity mechanisms, which proposed a role of Langerhans cells. T lymphocytes could be involved in induction of the eczematous response and also production of IL-4. [18,19]. The role in activation of B cell to produce IgE, IL-4 may be involved in increases the expression of FcεRI to amplify the response to aeroallergens. Strong IgE association with inhalant allergens are relevant to the pathogenesis to atopic dermatitis. [20,21] . Candida albicans, Cladospirm, Aspergillus, Pencillium and other air borne molds, have many antigenic components like proteins and polysaccharides. They induce Th-1, Th-2 dependent immune responses if they break through the skin. The patients with asthma show a shifting from Th1 and Th2 dominance of the immune response to the allergen stimulate Th1 causeing contact sensitity. Fungal allergy in asthma patients are aggravating factor.

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