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Antigen Specific IgG4 in Patients with Gastrointestinal Complaints

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Authors' contributions

This work was carried out in collaboration between all authors. Author VP designed the study, managed the analysis of the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author JN selected patients for study. Authors NK and JK managed the study from scientific point of view. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To find the antigen specific IgG4 in patients with gastrointestinal complaints and in control group, to demonstrate suitability of detection of IgG4 by using antigen panel.
Place and Duration of Study: Laboratory Management and Consultancy, Riga, Latvia, October 2012 - February 2013.
Methodology: The study included 147 patients (46 men, 101 women, aged from 1 to 76) with different complaints regarding gastrointestinal habitus. Antigen specific IgG4 analysis by using regional adopted antigen panel was executed.
Results: Almost all patients with gastrointestinal complaints -141 out of 147-have at least 2nd level antibodies, while in control group 15 out of 24 have 2nd level of antibodies.
Conclusion: IgG4 antigen specific antibody tests by panels could be used as a screening tool for food intolerance detection.

Keywords: Antigen specific IgG4; food intolerance.

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1. INTRODUCTION

Food hypersensitivity is a wide-spread and common problem, however, at the same time this issue has not been widely reflected in literature, except IgE class antibody and “true” allergy studies.

It is known that IgE class of antibodies sign the allergy in human body and antigen specific IgE as an allergy marker is determined worldwide. Since allergic reactions could be life threatening, allergist try to reverse IgE class response to IgG4 class response. As a result, IgG4 class antigen specific tests are developed for detection of successful immunological answer switch [1]. When IgG4 class antibodies are detected against particular “dangerous” antigen, allergists lost their interest in patients because their patients seem to be safe from anaphylaxis. IgG4 has been postulated to have a role in developing tolerance against allergens and in responses to certain infectious agents, but its physiologic role is poorly understood. In terms of tolerance it is always suggested as absence of IgE mediated allergic reactions and it is not taken into account less prominent, less visible IgG4 accompanied manifestations which in long term could also be harmful.

True prevalence of food intolerance is still unknown, but it has been estimated to be 5-7% and even reach up to 20% in general population. It is clear that IgG4 is not a cause of food intolerance but rather a result or mediator of adverse reactions to some food ingredients.

For a long time IgG4 has been considered as something normal or at least harmless. During the past few years we could find a lot of publications regarding IgG4 as a marker of Type 1 autoimmune pancreatitis (IgG4-related pancreatitis) and IgG4-related sclerosing cholangitis in medical literature and a lot of other serious conditions related to IgG4 class antibody presence in serum and tissues as well [2-8]. However, it is still little known about IgG4 formation or function in human body. Mainly for antigen-specific IgG4 investigations an IBS (Irritable Bowel Syndrome) patients probably were chosen due to the absence of any other explanations for such condition. Sameer Zar et al. described the correlations of antigen-specific IgG4 presence and food intolerance in IBS patients. As authors mentioned, in case of IBS - IgE class antibodies do not play pathophysiological role [9].

A very little information exist in scientific papers about IgG4 antigen specific antibodies and their relationships with food intolerance. The authors of study identify IgG4 antigen specific antibodies against most widely used products and compare the existence of such antibodies in healthy individuals and in individuals suffering from different gastrointestinal problems with the aim to find suitable laboratory test for detection of food intolerance against particular product. There is a high probability that food intolerance could be one of triggering factors for many pathologies. Improvement of physical conditions after exclusion diets show that some screening analysis should be helpful in selection of appropriate diet to avoid unnecessary dietary restrictions.

2. MATERIALS AND METHODS

In study was analyzed patient serum (n=147, female n=101, male n=46, mean age 24,6 ±17,1) and serum from healthy individuals as control (n=24, female n=18, male n=6, mean age 35,1±13,9) regarding presence of antigen specific IgG4 antibodies. Patients visited physician's office from October 2012 till February 2013. Blood initially was drawn for regular biochemistry analysis and residue of serum samples was used for the study. Patients with

gastrointestinal discomfort, bloating, flatulence, periodical or permanent problems of stool (from diarrhea to constipation) were selected for analysis. These patients were mainly managed by family physicians during many years. On several occasions patients were sent to gastroenterologist, gastroscopies were performed with no findings or therapy was unsuccessful.

Patients having diet restrictions during past 6 months or being on immuno-suppressive medications were excluded from investigation. Patients with suspected reaction to sugar components of vegetables – cabbage, apple, grape - were excluded since they could easily identify vegetables causing problems/symptoms themselves.

Serum from healthy individuals, who visited physician occasionally, was used as control. Specially composed antigen panels from Mediwiss diagnostic, Germany (commercially available) were used. They included 30 products being the most widely used in everyday diet in Latvia. Antigen panels and other reagents were provided by manufacturer as a whole kit and analysis was performed in accordance with manufacturer's recommendations.

Statistical analysis was done by PSPP program (free access). We used modified t-test for independent samples which take in count non-normal distribution of antibody levels. A probability level of <5% was considered to be statistically significant.

3. RESULTS AND DISCUSSION

Patients n=147 (male 46(31%), female 101(69%), mean age 24,6±17,1), control n=24 (male 6 (25%), female 18(75%), mean age 35,1 ±13,9).

The results of IgG4 antigen specific antibodies were divided in 6 classes – 0.00-2.90 µg/dl – 0 class (not detectable), 3.0-5.9 – 1st class (low threshold), 6.0-14.9 – 2nd class (increased), 15.0-59.9 – 3rd class (significantly increased), 60.0-179.9 – 4th class (high), 180.0-299.9 – 5th class (very high), >300.0 – 6th class (extremely high). Due to results of test reports given as values >300µg/dl in case of very strong reaction, we use classes for statistical calculations. Statistical analysis show significantly less overall reactivity and less formation of IgG4 class antibodies in control group - almost all patients with gastrointestinal complaints -141 out of 147 have at least 2nd class antibodies, while in control group 15 out of 24 have 2nd class antibodies. At the same time there is a significant reactivity by means of elevated IgG4 class antigen specific antibodies against cheese mix (M= 0.18 , SD=0.48 versus M=0.00, SD= 0.00, p=.00), gluten (M=0.33 , SD= 0.83 versus M=0.08, SD=0.41, p=.02), oat flour (M=0.90, SD=1.06 versus M=0.25, SD=0.53, p=.00), buckwheat (M=0.16, SD= 0.68 versus M=0.00, SD=0.00, p=.01) maize flour (M=0.22, SD=0.72 versus M=0.04, SD= 0.20, p=.02), wheat flour (M=2.01, SD= 1.87 versus M=0.92, SD=1.50, p=.01), rye flour (M=1.86, SD=1.85 versus M=1.08, SD=1.69, p=.05), banana (M=1.65, SD=1.59 versus M=0.46, SD=0.98, p=.00), egg white (M=3.01, SD=2.09 versus M=1.38, SD=1.28, p=.00), egg yolk (M=0.23, SD=0.73 versus M=0.00, SD=0.00, p=.00), whole milk (M=2.76, SD= 1.86 versus M=0.88, SD=1.26, p=.00), alpha lactalbumin (M=2.24, SD=2.54 versus M=0.71, SD=1.60, p=.00), beta lactoglobulin (M=1.28, SD=1.67 versus M=0.13, SD=0.45, p=.00), casein (M=1.31, SD=1.47 versus M=0.42, SD=0.72, p=.00) in patient group.

In some cases reactivity against rye, wheat proteins and gluten was discovered at the same time. In few cases strong reactivity against milk proteins, rye and wheat was detected at similar high levels.

Reactivity against casein was low compared to alpha lactalbumin and beta lactoglobulin. No statistically different reactions against meat (chicken, beef, pork), apple, oranges, carrot, celery, potato, fish (salmon, cod fish) in both patient and control groups were detected.

The strongest reactions were found against egg white (60% of patients), whole milk (54% of patients), alpha lactalbumin (44% of patients), wheat flour (40% of patients), rye flour (37% of patients), banana (37% of patients)– belonging to classes 3 to 6. Most 6th class reactions were against alpha lactalbumin, egg white and whole milk. All patients with detectable levels of antibodies against cheese mix do have antibodies against casein, but few patients with antibodies against casein show reactivity against cheese mix. The data representing IgG4 antigen specific antibody frequencies is shown in Table 1.

All cases of detectable IgG4 class antibodies against gluten exhibit significant reactivity against wheat and rye flour.

A lot of patients demonstrate surprisingly high reactivity to banana and almost all cases were accompanied with high levels of antibodies against egg white.

The results show high reactivity against products that many people consume in daily meals – milk proteins, wheat, rye, oat, egg white.

As it could be supposed, patients with the antibodies against whole milk show almost the same reactivity against small milk proteins. In many cases of such reactivity no antibodies against casein or cheese was found. In only one case isolated antibodies against casein were discovered. The reason could be the absence of enzymes for proper digestion of fresh milk. However, these patients demonstrated good tolerance towards milk products prepared by fermentation - cottage cheese, cheese, yogurt, kefir. In cases the antibodies against casein were detected, the patients mentioned feeling discomfort caused by consumption of any milk product.

In very few cases of reactivity against wheat and rye, reactivity against gluten on a much smaller scale is detected. It might be caused by individual peculiarities -reaction of organism to different proteins of the same products, however the development of coeliac disease in future for such individuals cannot be excluded. Likewise, the possibility that the first antigen in development of coeliac disease could be other besides of gluten/gliadin could not be rejected.

In many cases when serum demonstrated strong reactivity against milk proteins, rye and wheat proteins at the same time it was not possible to distinguish which antigen was most irritating.

In a few cases very strong reaction against 6-7 proteins simultaneously was detected. Mostly such reactions were observed in children (less than 10 years old) with long history of unresolved gastrointestinal problems. Many of them had a typical clinical picture of coeliac disease, but not responded on gluten free diet.

During two last decades social and economic situation in Latvia has changed and the range of products for daily diet even for infants, has widened. Banana is among widely used products, as it could be easy prepared and introduced as a first mild product in infants diet. Consequently, it is common to detect antibodies against banana. However, at the same time these patients had high levels of antibodies against egg white. It could be speculated that

banana proteins – relatively alien for population- given young age could irritate immature intestine and raise the antibody formation against egg white, which is introduced later.

Table 1. IgG4 antigen specific antibody frequencies by classes

	0 class		1-st class		2-nd class		3-rd and higher class	
Antigen source	control (%)	patients (%)	control (%)	patients (%)	control (%)	patients (%)	control (%)	patients (%)
gluten	23 (96)	122 (83)	0	10 (7)	1 (4)	7 (5)	0	8 (5)
cheese mix	24 (100)	127 (86)	0	15 (10)	0	4 (3)	0	1 (1)
oat flour	19 (79)	79 (54)	4(17)	16 (11)	1 (4)	40 (27)	0	12 (8)
maize flour	23 (96)	128 (87)	1 (4)	12 (8)	0	4 (3)	0	3 (2)
banana	18 (75)	59 (40)	3(13)	11 (7)	2 (8)	23 (16)	0	54 (37)
egg white	8 (33)	28 (20)	6 (25)	11 (7)	4 (17)	21 (14)	5 (21)	88 (60)
egg yolk	24 (100)	132 90)	0	2 (1)	0	8 (5)	0	5 (3)
wheat flour	16 (67)	50 (34)	2 (8)	13 (9)	1 (4)	26 (18)	5 (21)	58 (40)
rye flour	15 (63)	54 (37)	2 (8)	15 (10)	1 (4)	25 (17)	6 (25)	53 (37)
whole milk	13 (54)	22 (15)	5 (21)	17 (12)	4 (17)	30 (20)	2 (8)	78 (54)
alpha-lactalbumin	19 (79)	73 (50)	1 (4)	5 (3)	0	6 (4)	4 (18)	63 (44)
beta lactoglobulin	22 (92)	82 (56)	1 (4)	9 (6)	1 (4)	17 (12)	0	39 (27)
caseine	17 (71)	61 (42)	4(17)	21 (14)	3 (13)	45 (31)	0	20 (14)
buckwheat	24 (100)	136 (93)	0	5 (3)	0	3 (2)	0	3 (2)
bell pepper	21 (88)	127 (86)	0	4 (3)	1 (4)	11 (7)	2 (8)	5(3)
soya	24 (100)	137 (93)	0	5 (3)	0	4 (3)	0	1(1)
pea	24 (100)	145 (98)	0	1(1)	0	0	0	1(1)
peanut	24 (100)	134 (91)	0	3 (2)	0	4 (3)	0	6 (4)
coconut	24 (100)	144 (98)	0	1(1)	0	1(1)	0	1(1)
codfish	24 (100)	146 (99)	0	1 (1)	0	0	0	0
salmon	24 (100)	146 (99)	0	0	0	0	0	1(1)
carrot	24 (100)	142 (97)	0	2 (1)	0	1(1)	0	2 (1)
celery	24 (100)	137 (93)	0	5 (3)	0	2(1)	0	3 (2)
apple	23 (96)	131 (89)	0	4 (3)	1 (4)	5 (3)	0	7 (5)
orange	24 (100)	135 (92)	0	3 (2)	0	4 (3)	0	5 (3)
beef	24 (100)	147 (100)	0	0	0	0	0	0
pork	24 (100)	140 (95)	0	5 (3)	0	2(1)	0	0
chicken	24 (100)	143 (97)	0	0	0	4 (3)	0	0
potato	24 (100)	140 (95)	0	3 (2)	0	1(1)	0	3 (2)
tomato	24 (100)	141 (96)	0	4 (3)	0	1(1)	0	1 (1)

During the study there was a significant number of patients with antibodies against oat proteins that could be caused by traditionally high exposure to oat proteins in early age, however, antibody levels are not so high as in case of wheat and rye.

High reactivity against soy was expected but it was not detected. People being suspicious of some reactivity against milk proteins start to replace animal and human milk with soy milk in

their or their children's diet. Soy has been increasingly used in food manufacturing, and as soy is a foreign product for Latvia's population, the presence of antibodies against soy can be supposed. However, results show very rare cases and low levels of antibodies.

It would be more notable to find any antibodies against pork, beef, chicken meat, salmon, cod fish. It can be supposed that products which are more difficult to digest (e.g., fibres) or proteins of these products which have no enzymatic activity *per se* pass through intestines in much more harmless way and do not initiate IgG4 class antibody production.

The possibility that some antigens in antigen panels used in the research were missed cannot be excluded. Due to the use of the same antigen compositions as for IgE class antibody detection, the possibility that IgG4 class antibodies *in vivo* could be synthesized against different epitopes of the same protein cannot be excluded, and, consequently, it was not possible to detect them.

Detection of several antigen-specific IgG4 at the same time using adopted panel is the most convenient due to low costs and information acquired. During a single test is possible to check antibodies against 30 antigens and acquire more or less complete picture regarding reactivity against particular antigens or antigen composition. In case of antibody detection in single test, e.g. against milk only as a whole, it is not possible to identify which antigen is most irritating and a lot of other antigens could be missed.

During the study, three main problems can be identified. First, it is difficult to find healthy individuals for control group. Often people do not recognize themselves as persons having any problems. Only after direct questions about bloating, reflux, burden in stomach they admit taking some medication to improve digestion.

Next, it is not easy to provide right recommendations regarding diet and options to avoid problematic product. Even in the case of reactivity against only one antigen, it is almost impossible to avoid any challenge with product containing it. Milk proteins are widely used in hidden ways – in bakery, sausages and snacks. The same is true in case of wheat proteins and egg white.

Third problem is follow up – IgG4 class antibodies could persist a long time and even a small antigen exposure could give impulse for antibody synthesis, thereby repetitive detection of IgG4 antigen specific antibodies is not a suitable test for follow up purposes.

There is a high probability that long time exposure to antigens which cause permanently high level IgG4 synthesis could be the triggering factor for some autoimmune or even malignant disease development.

After execution of the study the following questions might be raised: why do we have not these class antibodies against a lot of antigens we meet every day during our life? Is it dangerous to have a high level of IgG4 to particular antigen? Could this "particular" level of antigen-specific antibodies form a high level of total IgG4? What happens with people having high level to specific antigen – are they symptomatic in any way before they get so symptomatic to develop any serious disease? Could this antigen specific IgG4 turn into auto-reactive antibody type as it do in case of coeliac disease?

4. CONCLUSION

After having done the study, the following conclusions can be singled out. IgG4 antigen specific panel test kits are suitable for food intolerance detection for patients with gastrointestinal complaints.

IgG4 antigen specific panel test kits are relatively cheap and easy to use for well-trained laboratory staff. The amount of serum needed for one panel is 25 µl therefore this test could be used even in cases if small sample is available, specially – in children.

It is not necessary to detect IgG4 level precisely, as it could not be used as a test for follow up or measurement of patient's hospitality.

It is possible to compose antigen panels according to traditionally used food for every country on request of regional laboratory. In case some exotic antigen is suspected the single particular antigen test could be performed separately.

The questions about food intolerance and coeliac disease are still open since not all coeliac patients react well on gluten free diet and many of them have IgG4 class antibodies against other antigens.

The gastrointestinal complaints occur not solely in case of food intolerance. The symptoms could be atopic dermatitis, asthma, frequent cold, headaches, problematic skin, hair loss and many other, consequently further investigation is needed on antigen specific IgG4 link with these symptoms.

CONSENT

Informed consent was obtained from adult patients or from the children's parents.

ETHICAL APPROVAL

The study was approved by the Ethics Committee at the Riga Stradins University.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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