Original article

The first report on immunoglobulins A, E, G and M levels in cystic fibrosis patients in Saudi Arabia

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ABSTRACT

Background: Previous reports have shown that pulmonary and systemic hypergamma-globulinemia in CF patients is a reflection of chronic pulmonary infection. Infection with *Pseudomonas aeruginosa* is known to have major prognostic significance in patients CF. This study aims to identify the incidence of immunoglobulins (especially: IgG, and IgE) in a cohort of CF patients.
Methods: A total of 297 patients recruited all over the country's region for this study were a as part of the CF registry data from 1st January 1984 to 1st June 2016. All patients had their immunoglobulin levels measured by enzyme link immunosorbent assay (ELISA) in 3 stages, at presentation and two follow-ups.
Results: Of the 297 patients recruited, 139 (46.8%) were males while 158 (53.2%) were females. IgA and IgM levels were found not to have risen above the previously reported levels in healthy individuals in all stages. On the contrary, IgE level increased from 209.51 ± 32.30 KU/L to 303.58 ± 37.11 KU/L from baseline to stage 3 while IgG level rose from 12.26 ± 0.43 mg/mL to 17.17 ± 1.68 mg/mL for baseline and stage 3 respectively all above previously reported levels in healthy individuals.
Conclusion: This study establishes a potential for the use of IgE and IgG in disease diagnosis as well as the prognostic implications. However, further study is needed to identify the role of infection or medications in relation to the rise of both IgE and IgG with advancement of age and progression of disease severity which may inherently confound the observed results.

1. Introduction

Cystic fibrosis (CF) is a chronic infectious disease of the lungs and airways that develops due to defect in the cystic fibrosis trans-membrane conductance regulator (CFTR), which conducts transport of chloride ion (Cl^-) and bicarbonates (HCO_3^-) across the cell membrane in epithelial cells (Tang et al., 2009). This defect results in reduced fluid transport to the airway epithelia and increased mucus secretion. In turn, the epithelial surface becoming sticky exhibiting reduced mucocilliary clearance. As such, trapped bacteria such as *Pseudomonas aeruginosa* colonise the epithelia inducing

development of bronchiectasis with progressive disease that often lead to respiratory failure in both children and adults globally (Redondo et al., 2016).

Pseudomonas aeruginosa is a major pathogen in opportunistic and nosocomial infections, consequent of its inherent nature to develop multi-drug resistance to various antibiotics and its infection is the major cause of the complications in CF patients often resulting in death. As a defence mechanism, studies have shown that CF patients, over time raise immunoglobulins against different bacteria especially *Pseudomonas aeruginosa* and *Staphylococcus aureus* in a condition known as hypergammaglobulinaemia to combat the infection (Baldan et al., 2014). While there are very few studies that have reported secretion of IgA in response to *Pseudomonas aeruginosa* (Mauch et al., 2017; Aanaes et al., 2013; Hansen et al., 2012); the correlation of the secretion with disease progression and prognosis has not been studied. IgA is secreted as a humoral response against bacterial toxin or viral particles preceding activation of neutrophils for instance, which is required for protection of mucosal surfaces to prevent epithelia damage by pathogens. This might have been the reason why Aanaes, Johansen (Aanaes et al.,

2013), Hansen, Rau (Hansen et al., 2012) and Mauch, Rossi (Mauch et al., 2017) were able to record IgA secretion in airways of CF patients in response to *Pseudomonas aeruginosa*. Reports on IgM secretion in CF is even more scanty. This may be attributed to the fact that IgM is mostly involved in B cell development and opsonisation of antigens (Schroeder and Cavacini, 2010), which is an area that is yet to be pursued in CF with respect to IgM involvement. Most other studies have reported significantly high levels of IgE and IgG in CF patients in response to different bacteria and even fungal infections (Table 1). Patients with chronic infections are found to have extremely high levels of both IgG and IgE compared to non-infectious CF patients or healthy individuals. In addition, CF patients are found to be susceptible to provoked immune response as found in those that are sensitive to *Aspergillus fumigatus*. As a result, such patients also produce significantly high level of immunoglobulins including IgG and IgE in response to the allergen.

Conventional diagnosis of CF involves examination of patient sputum which may be difficult to provide by a child patient, or broncho-alveolar lavage which is too invasive, for bacterial culture. Based on these, several studies have posited the use of IgG and IgE levels as a diagnostic and prognostic tool for determining disease progression and overall survival of CF patients (Vitte et al., 2017). In fact, some of the criteria of Cystic Fibrosis Foundation Consensus Conference for diagnosing allergic bronchopulmonary aspergillosis (ABPA) in CF and asthma patient include having serum total IgE level above 1000 IU/mL as well as presence of anti-Aspergillus IgG in the serum (Chotirmall et al., 2008). ABPA is known to develop in these patients due to immune response to *Aspergillus fumigats*, a fungus that is commonly found in nature. However, in CF patients, a rise in IgG or IgE levels is not uncommon in response to *Aspergillus fumigatus* even when the patient does not present with ABPA (Chotirmall et al., 2008). In addition to all these, an unpublished data from a retrospective study carried out in our lab over the period of 33 years showed that CF patients presents with significantly higher levels of IgG and IgE irrespective of their age when compared with normal patients.

Various factors as stated above and presence of allergens can trigger increased immune response in immunodeficient patients especially CF patients. This hints at the benefit of employing IgG and IgE levels as diagnostic factor in CF patients for quick diagnosis of such patients. While it is not fully understood whether allergens or infection alone accounts for the increased level of the immunoglobulins observed in these patients, other factors such as the type of medication and immune status of patients may also contribute. In addition to this, it has also been posited that the increased level of immunoglobulin in the airway epithelial may result in autoreactivity with the antibodies attacking the epithelial layer of the airways and the lungs further aggravating the disease (Wallwork et al., 1974). This study thus aimed to investigate the potential of IgG and IgE levels as a rapid diagnostic and prognostic tools in patients with CF in Saudi Arabia.

2. Materials and methods

This is a retrospective chart review as part of the CF registry data from the period 1st January 1984 - 1st June 2016. The ethical approval for the study was approved by the Research Ethical Committee of King Faisal Specialist Hospital and Research Centre in Saudi Arabia with reference number ORA/1179/37. Immunoglobulins A, E, G and M were quantified in all confirmed CF the patients for all age groups at baseline (stage 1) and two follow ups at stages 2 and 3 at time intervals shown in Table 3. Serum from patient

Table 1
Summary of studies showing significant IgG and IgE secretion in CF patients.

Study	£ Age (years)	Sample size (Test/healthy control group)	$ IgG (normal)	$ IgE (normal)	Infection	P value
* Mauch et al. (2014) (25)	14.7 (median)	117/53	96.8% (1.89%)	NA	chronic or intermittent *P. aeruginosa*	<0.0001
	9.0 (median)		33.3% (1.89%)	NA	infection	<0.0001
	8.2 (median)		11.1% (1.89%)	NA	infection free	<0.0001
	3.0 (median)		2.9% (1.89%)	NA	non-colonised	<0.0001
& Clerc et al. (2017) (26)	29 ± 9 (mean)	43/122	228 ± 68 mg/dL (55 ± 34)	NA	intermittent *S. aureus* /*P aeruginosa* colonisation (*S. aureus*)	<0.0001
Hodson et al. (1988) (27)	NA	32/27	277 IU/mL (1 6 5)	70 IU/mL (30)	N/A	<0.001 IgG <0.005 IgE
Skov et al. (1999) (28)	18 (median)	238/107	NA	NA	chronic and intermittent *A. fumigatus* colonisation	<0.001 IgG
Knutsen et al. (1994) (29)	3–6	65/NA	NA	NA	chronic *A. fumigatus* infection	<0.01 IgG <0.01 IgE
Wallwork et al. (1974) (10)	3–5	59/NA	NA	275 IU/mL (65)	NA	NA
	5–10		NA	1030 IU/mL (79)	Chronic infection	NA
	10–15		NA	828 IU/mL (1 0 2)	Chronic infection	NA
Murali et al. (1994) (30)		14/10	0.324 EU (0.03)	0.223 EU (0.264)	*P. aeruginosa* and *A. fumigates infection*	<0.01 IgG IgE ns

$ Values for normal patient in bracket.
* Study considered percentage of patient group that are seropositive.
£ Age statistic indicated in parenthesis.
& Study considered IgG concentration.
ns = not significant.
EU is ELISA unit.

blood samples were collected following previously published procedure (Shead et al., 2010). Briefly, peripheral blood collected from patients were added into serum-gel monovettes (Sarstedt, UK) and mixed well, then allowed to clot. Serum was frozen at −80 °C within 2 hours of separation or used for enzyme linked immunosorbent assay (ELISA). The serum immunoglobulins levels were all measured using ELISA kit.

2.1. Immunoglobulin quantification

IgM, IgG and IgA class antibodies against *Pseudomonas aeruginosa* PAO1 strain, were analysed principally as described earlier. The polystyrene microtitre plates (Nunc, Roskilde, Denmark) were coated with extracts of *Pseudomonas aeruginosa* PAO1 flagellin antigen (5 mg/ml) in phosphate-buffer saline (PBS; 0.1 mol/l, pH 7.5; 100 ml/well) overnight at 37 °C. The plates were saturated with 1% bovine serum albumin in PBS (BSA ± PBS; 100 ml/well). Patient serum samples extracted above were diluted at 1:250 for IgM, IgE and IgA or 1:300 for IgG (75 ml/well) were incubated on the plates for 2 hours at 37 °C. Afterwards, 75 ml/well of alkaline phosphatase-conjugated swine anti-human IgA, IgE, IgG or IgM were diluted at 1:250, 1:250, 1:250 and 1:500 respectively, and were incubated on the plates overnight at room temperature. Fresh phosphatase substrate (1 mg/ml of p-nitrophenyl phosphate in diethanolamine, $MgCl_2$ buffer solution was added. The plates were incubated for 30 min at 37 °C and the reaction stopped with 1 M sodium hydroxide. The optical density was measured with a Bio Tek EIA Autoreader Model EL310 at a wavelength of 405 nm.

IgA, IgG and IgM were measured by a single radial immunodiffusion, a previously described method (Dunn et al., 2018), using a commercial kit (Kallestad Laboratories, Inc., Minnesota, USA), following manufacturing instructions. Serum IgE measurements were carried out using PRIST technique using commercially available kits from Pharmacia Diagnostics (Uppsala, Sweden). All samples were assayed in triplicate within the same batch.

2.2. Statistical analysis

All data collected were in triplicates and the mean and standard deviations were computed using Microsoft Excel 2016.

3. Results

3.1. Patient demographics

For this study, a total of 297 patients were recruited, 53.2% (158) of whom were female while 46.8% (139) were male patients. Of these patients, 38% (113) were from the west of Saudi Arabia, 24.2% (72) from the central region, 15.5% (46) from the northern region and 11.1% (33) each from the western and the southern regions.

3.2. The measure of the IgA level in the three stages

Quantification of IgA levels in the patients was carried out in three stages. In the first stage, which was at the baseline of the study, mean IgA levels measured in 156 patients was found to be 1.9 ± 0.16 mg/mL. In the second stage, which occurred after a mean period of 25 months, IgA was quantified in 6 of the patients and the mean level was found to have slightly dropped to 1.74 ± 0.67 mg/mL while in the last stage which occurred after a mean period of 30.8 months from the second stage, the IgA levels measured in 79 patients was found to increase to 2.023 ± 0.15 mg/mL (Table 2 and 3). According to the lower and upper limit ranges as well as the mean value that were quoted for IgA in a study by Harfi and God-

win (Harfi and Godwin, 1985) that quantified IgA in normal and health Saudi Arabia adults, the mean IgA level was measured to be 2.17 mg/mL which was higher than the levels measured here in all the three stages.

3.3. The measure of the IgE level in the three stages

For IgE, the mean level measured in 272 patients at the first stage which was baseline measurement was found to be 209.51 ± 32.30 KU/L. In the second stage where the IgE level was measured after a mean period of 38.37 months from the baseline measurement, this level increased to the highest level measured at 305.86 ± 52.24 KU/L as quantified in 160 patients. In the last stage which occurred after a mean period of 53.98 months from the second stage, the mean IgE level slightly dropped to 303.58 ± 37.11 KU/L (Table 2 and 3). Interestingly for IgE, the mean value reported here was higher more than two folds that of the reported normal values by Harfi and Godwin (Harfi and Godwin, 1985) (90.8 kU/L) for all the three stages.

3.4. The measure of the IgG level in the three stages

As with the other immunoglobulins, IgG was also measured in three stages. In the first stage occurring at the study baseline, the mean IgG level measure in 212 patients was found to be 12.26 ± 0.43 mg/mL which increased to 15.18 ± 1.55 mg/mL quantified in 96 patients after a mean period of 27.6 months from baseline measurement. This further increased to 17.17 ± 1.68 mg/mL quantified in 142 patients in the third stage after a mean period of 42,29 months from the second stage measurement (Table 2 and 3). According to the mean value of IgG reported by Harfi and Godwin (Harfi and Godwin, 1985) (11.67 mg/mL), the IgG levels reported here are slightly higher at all the three stages.

3.5. The measure of the IgM level in the three stages

IgM levels were also quantified in the patients in three stages as above. In the first stage which occurred at baseline, the mean IgM level measured in 161 patients was found to be 1.17 ± 0.05 mg/mL which increased to 26.65 ± 25.91 mg/mL in the second stage as measured in only 8 patients where the measurement was taken after a mean period of 11.33 months from baseline and 1.07 ± 0.04 mg/mL in the third stage as measured in 68 patients where the measurement was carried out after a mean period of 25.125 months after the second stage (Table 2 and 3). Just as for IgA, the mean level of IgM measured in this study was also similar to that reported by Harfi and Godwin (Harfi and Godwin, 1985) which was 1.67 mg/mL. This was less than the mean value reported in the first and last stage except for the second stage where there was almost 16 folds the normal value by Harfi and Godwin (Harfi and Godwin, 1985).

3.6. Patients presenting with (ABPA)

To access the effect of ABPA as a confounder in the immunoglobulin measurements carried out, a survey was carried out on all patients to find out which of them were ABPA patients. Of the 297 patients that were included in this study, only 10 (3.4%) reported to present with ABPA.

4. Discussion

Secondary infection and inflammation due to pathogens like *Pseudomonas aeruginosa* or allergens like *Aspergillus fumigatus* are the main cause of death in CF (Reece et al., 2018; Banjar, 2003).

Table 2
Descriptive statistics of immunoglobulin levels at baseline and follow ups.

Immunoglobulin type (normal level)/mean	At presentation		At followup#1		Last follow up	
	Number of patients	Mean (SD)	Number of patients	Mean (SD)	Number of patients	Mean (SD)
IgA (0.5–5.9)/2.17	156	1.9 (2)	6	1.74 (1.63)	79	2 (1.34)
IgE (1.5–489)/90.8	272	209.5 (32.3)	160	305.86 (52.23)	230	303.57 (37.1)
IgG (5–17.8)/11,67	212	12.3 (0.4)	96	15.18 (1.55)	142	17.1 (1.68)
IgM (0.32–5.1)/1,67	161	1.17 (0,05)	8	208 (26.65)	68	1.07 (0.05)

(SD) = Standard deviation.

Table 3
Descriptive Statistics of period in months for all immunoglobulins.

Immunoglobulin type (normal level)/mean	First and second		Second and third		First and last follow up	
	Number of patients	Mean (SD)	Number of patients	Mean (SD)	Number of patients	Mean (SD)
IgA	11	25.4 (9.7)	11	30.8 (7.72)	75	64.97 (5.13)
IgE	163	38.37 (2.36)	162	53.98 (2.83)	232	78.38 (3.45)
IgG	11	27.6 (9.48)	96	63.45 (3.93)	141	63.45 (3.93)
IgM	9	11.33 (1.72)	8	25.13 (6.66)	63	65.27 (6.17)

(SD) = Standard deviation.

Although it early mortality in CF is considered to be multifactorial, which may be due to complications from infections, allergic reactions, and immunodeficiency (Banjar, 2003), early diagnosis that will aid proper disease management may help improve the patients' overall survival. The innate immune system responsible for secretion of immunoglobulins such as IgE and IgG is the primary defence system of CF patients against these pathogens. As such, the level of these immunoglobulins may be indicative of the stage of infection or disease of in a patient with CF. To the best of our knowledge, this is the first study that quantifies the levels of IgA, IgE, IgG and IgM in a cohort of adult patients presenting with CF. Findings from this study produces useful insight into the importance and potential of IgE and IgG levels as diagnostic and prognostic tool in CF patients for early and accurate evaluation of patients CF status.

Hypergammaglobulinemia involving IgG and IgE have long been associated with CF especially in patients with aggravated chest and lung infections as well as APA (Moss, 1987; Marchant et al., 1994). In an attempt to investigate the incidence of hyperglobulinaemia and possibility gamma globulins application as diagnostic tool in CF, we measured and reported here that the mean level of IgE increased dramatically in the second stage (209 kU/L to 305 kU/L), and this immunoglobulin level was maintained at the third stage. This level was 16 folds higher than that of the normal values previously reported in normal healthy Saudi adults for period spanning 78 months (Harfi and Godwin, 1985). Likewise, the IgG values that were reported here in all three stages were slightly higher than the mean values that have been reported by Harfi and Godwin (Harfi and Godwin, 1985). Ortega-López, Escobar Quintero (Ortega-López et al., 2016) quantified IgG and IgE in levels in patients with CF and found out that 34.2% and 41.4% of the patients had IgE and IgG levels that were above the normal range considered for the study. A recent study carried out in a cohort of CF patients also indicated the IgG level of patients continued to increase as the disease progresses as long as the patient continue to age alongside aggravated chest infections (Proesmans et al., 2011). In addition to this, Ortega-López, Escobar Quintero (Ortega-López et al., 2016) indicated that the patients with high IgE levels are those with allergic sinusitis, allergic rhinitis, nasal polyps, and APA. As such in response to chest infection that is characterised with bacterial colonisation of the airways and lungs involving for instance, Pseudomonas aeruginosa, IgG quantification may serve as a useful diagnostic tool. This is likely because IgG is required for the opsonisation of bacteria such as Pseudomonas aeruginosa during infection through the binding of the Fc region of the IgG to the bacterial surface proteins to facilitate easy phagocytosis of the bacteria (Nordenfelt et al., 2012). In the same manner, IgE levels can be indicative of disease progression due to sensitisation to allergens such as Aspergillus fumigatus. Binding of allergens such as Aspergillus fumigatus by macrophages and dendritic cells initiates a complex intracellular process that culminates in the clonal expansion of B cells and production of antigen specific IgE which eventually binds mast cells causing its degranulation and release of pro-inflammatory molecules like prostaglandin 2 (PGD2), histamine and tumour necrosis factor (TNF). This thus induces the perpetual inflammation that is often observed in patients with CF in response to allergens (Galli and Tsai, 2012).

On the contrary, the mean values reported for both IgA and IgM were less than that reported by Harfi and Godwin (Harfi and Godwin, 1985). Although the IgM level recorded in this study at the second stage was 20-fold that recorded at baseline and the third stage, this may have been confounded by the fact that only eight patients were available for IgM measurement compared with the 161 and 68 patients in the first and last stage respectively. This is an indication that the IgA and IgM levels that were recorded in this study is not influenced by the stage of the disease or whether the patients present with APA. This is in agreement with the finding of some studies that have measured IgA and IgM levels in CF patient. Collin, Detry (Collin et al., 2017) reported no significant difference in serum IgA and IgM levels between CF patients and normal individuals used as control groups. In the same manner, Ortega-López, Escobar Quintero (Ortega-López et al., 2016) reported only 17.1% of the patients having IgA and IgM levels that were higher than the normal levels considered in their study. This was significantly lower than the percentage of patients that had above normal levels of IgG and IgE. There was however, a report of elevated serum IgA and IgM with respect to normal individuals. Bernardi, Ribeiro (Bernardi et al., 2013) reported a significantly higher IgA and IgM level in CF patients. This may be because these were paediatric patients with differing immune response to adults that were recruited in this study. Interestingly, Ortega-López, Escobar Quintero (Ortega-López et al., 2016) also reported IgA levels to be higher in paediatric patients as reported by Bernardi, Ribeiro (Bernardi et al., 2013). This could be an indication of high IgA and IgM levels in paediatric patients compared with adult patients suffering from CF. The high IgM level in stage 2 may also attributed

to the small number of patients from which the samples were collected, resulting in report on few patients having overall high IgM levels.

Patients with CF suffer from a host of bacterial infections that is not limited to *Pseudomonas aeruginosa*. Some of these infections is caused by opportunistic bacterial like *Staphylococcus aureus*, non-tuberculous mycobacteria and *Haemophilus influenzae* all of which induces substantially high production of IgG. Although, the type of bacterial infection in the patients were not investigated in this study, there are studies that have reported the common occurrence of these bacterial infection in CF patients (Bernardi et al., 2013; Spasenovski et al., 2010). As such, a time dependent rise in IgG level is expected in the CF patients as long as bacterial infection continue to aggravate.

While data from this study shows increase in IgG and IgE levels making these two immunoglobulins a good prognostic biomarker for disease progression in CF patient, there is need for caution in interpreting the result of IgM measurement due to the possible confounding factor in the small number of patients in which measurements were taken in the second stage, giving rise to unusually high level of IgM in that stage. In addition to this, the observation that the prevalence of CF is higher in the central and eastern region of Saudi Arabia calls for concern. This may be likely due to increased genetic predisposition in these regions but further studies is required to ascertain this and possibly help to focus attention to these areas for better disease management.

5. Conclusion

The result from this study indicated that IgG and IgE levels in patients with CF rises with disease progression and advancement of age in contrast with IgA and IgM which either showed no increase with respect to normal levels. As such, the level of these immunoglobulin may serve as a tool to determine the disease stage in the patients for better planning of disease management and possible treatment modalities.

Declaration of Competing Interest

Authors declare no conflict of interest.

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