Medhat Elamawy's 2017 Clinical Fellowship Final Report

First of all, I would like to thank EAACI jury for giving me the opportunity to have this unique experience in clinical immunology and allergy department at king's college hospital, London. I appreciate the welcoming and friendly treatment of all king’s team specifically my host supervisor Dr Mohammad Ibrahim. I am also grateful to my home institution for putting me forward and home supervisors especially Dr Maged Refaat for his generous helps.

- EAACI Clinical Fellowship Awarded : Dr Medhat Elamawy
- Scholarship type: Clinical Fellowship
- Project title : Non Canonical NF kappa B signaling in patients with antibody Deficiency
- Home institution: Benha Faculty of Medicine, Benha University, Benha, Egypt
  Website: http://bu.edu.eg/en/
- Home supervisors: Dr Maged Refaat Professor of Allergy and clinical immunology
  Dr Mohammad shawki Professor of Internal Medicine
- Host supervisor: Dr Mohammad Ibrahim, Department Head, Consultant, and Clinical Lead.
- Venue: Clinical Immunology and Allergy Department at King's College Hospital, London, UK
  Website: https://www.kch.nhs.uk/
- Duration: 3 months
- Start date: Monday, 18 September 2017
- End date: Friday, 15 December 2017

Preparation and my first meeting:

I prepared myself for the clinical training before travelling by gaining as access to one of major textbooks in allergic field “Middleton's allergy principles and practice 8th Ed, Franklin Adkinson Jr.,
MD.” I am very grateful to Dr Adkinson and Elsevier Editor for offering me a complimentary copy in exchange for a review of the title. Also clinical attachment to Dr Maged Refaat in my thesis preparation was one of the best preparation tools. In addition to that I prepared myself for the project work by asking Dr Ibrahim to send me some relevant articles. In UK, The team was nice and so friendly. I had the nice welcoming first meeting with my Host Supervisor, Dr Mohammad Ibrahim, Department Head, Consultant, and Clinical Lead. I was completely happy that I was in the right place. We revised the objectives and put a schedule to get the most benefit of my time. One of the best advices I had in my life, he asked me to optimize my weeks training; one week for predominately research work alternates with a predominately clinical experience week. It was the essence of objectives focusing.

**My main project and learned skills:**

**My main project:**

**Context:** Common variable immune deficiency disorders (CVID) are considered the most common symptomatic diseases of Primary immunodeficiency diseases (PID) (1) CVID patients had varied clinical manifestations like recurrent infections, autoimmunity, malignancy, and lymphoproliferative disorders. These features reflect its heterogeneous nature. (2)

The genetic mutations comprise up to 20 % that usually have positive family history. (3) Non-canonical nuclear factor κ B (NF-κB) signaling pathway gene mutations represent an example for these genetic bases. (4,5)

Although alopecia and central endocrinal deficiency were common presentation in these patients, we still have not a unifying schematic approach to diagnosis NF-κB2 gene mutations. By fulfilling this,
we can follow up the index cases and relative family members efficiently and pick up the CVID cases that need more genetic processing. In this project, my roles were:

1. To Review all case reports and series that published on NF-κB 2 mutations in CVID patients, to tabulate their clinical characteristics whether common or rare, cultured pathogens if infections history found, and laboratory finding, and to find correlation between these finding to unify them. The lists were ended on 25/9.

2. To list clinical and laboratory finding of all 43 CVID patients that was found on king’s college hospital records aiming to get more discrepancies between this list and CVID patients with NF-κB 2 mutations list. The lists were ended on 15/11

3. To obtain a list of all non-canonical signal pathway genes mutations that linked with CVID patients through different methodological approach; of them to search by PubMed. I reviewed the search items thoroughly on 27/10 and the lists were ended on 24/11 with kind help of Dr Kruthika Sundaram Scientific Research Officer

These steps were under kind supervision of Dr. Mohammad Ibrahim. By which I gained more experience on how to formulate a research question efficiently, and how to prepare for a systematic review hoping to get EAACI Congress grant to present the full detailed work in 5/2018.

My learned laboratory skills: (my first time to do laboratory experiments)

The principles are to Shadow all scientists in their experiments, after getting the protocols and understanding it. I participated in some experiments then I had later discussion with them to get full benefit. Examples of these skills are:

1. Enzyme-linked immunosorbent assay (ELISA) and its other based assay like Trans AM NFκB Kits to study Nuclear Factor κB (NFκB) family member.
2. Different techniques for the isolation of human peripheral blood mononuclear cells (PBMCs) whether by Ficoll-Hypaque density gradient centrifugation or other comparable methods (I joined Terrence Hunter, Biomedical Scientist).

3. Diverse ways of expressing concentrations and dilution, how to get serial dilutions from stock solute, Micro Pipettes efficient use, counting chamber hemocytometer protocols, and ELISA Microplate reading equipment dealing skills.

4. MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) tetrazolium reduction assay for assessing cell metabolic activity. They study the effects of cellular stress on survival of B lymphocytes with NFκB mutations after cell culture (I joined Dr Kruthika Sundaram Scientific Research Officer).

5. Different Protein assays like Lowry protein assay and Bio-Rad DC Protein Assay which are colorimetric assays for protein concentration in a solution that measured later using spectrophotometry. (I joined Terrence Hunter for preparation a standard curve to quantify the amount of NFkB in our samples).

6. Flow cytometry: I learned the basic principles of this laser based technology and joined my colleagues Research and Clinical Fellows Helene Martini, in some steps in her isolation experiments of T-Follicular Helper (Tfh) cells to understand some of its biological properties and Ioasaf Karafotias, MD, MSc in his experiments on monocytes purification and staining in CVID patients so I learned a bit about the basics of sorting and compensations as well.

7. Some statistical analysis and graphical representation of data. I attended SIGMAPLOT Software lecture with Dr Mohammad Ibrahim and used Excel formulas in dealing my experiment results with Terrence Hunter.

8. Diagnostic laboratory skills; Mary Guckian, clinical scientist helped me to know how to perform total IgE and immunoCAP lab tests.
With Dr Mohammad Ibrahim supervision, generous help of Terrence Hunter and Dr Kruthika Sundaram, I used Trans AM NFκB Kits to study Nuclear Factor κB (NFκB) family member. The Kit combines a fast and easy ELISA format with sensitive and specific assay for NFκB transcription factors study. The objectives were to figure out the effects of CD40 L stimulation on NFκB2 gene mutation B lymphocyte cells.

By generating lymphoblastoid cell lines (LCL) of peripheral B Lymphocytes by Epstein - Barr virus (EBV) transformation and nuclear extraction, we worked on NFκB members (RelB, P50, P65, c-REL and P52) quantifications in 2 related subjects (as a case control experiment); one had normal non-canonical NFκB signal pathways with no NFκB2 gene mutation, the other had NFκB2 gene mutation. We stimulated both with CD 40 L. Finally we measured their optical densities with colorimetric reaction.

Being as positive control for NFκB activation, the Raji nuclear extract was supplied in the kits tool. The Wild Type and Mutant Type consensus oligonucleotides were provided as competitors for NFκB binding in order to monitor the specificity and sensitivity of the assay, respectively. I practiced pipetting and analyzing my results. I conducted these experiments with Terrence Hunter help, discussed my results and got feedback through lab meetings.

In the clinical immunology and allergy department, I attended two journal clubs and eight research meetings. In journal club, my colleagues presented research articles based upon proposed selection criteria. Dr Kruthika Sundaram presented the last interesting article that was entitled “Single-cell RNA-seq identifies a PD-1hi ILC progenitor and defines its development pathway”. (5) It was interesting to attend this event as it helped me in critically evaluation relevant international articles in the scientific literature. In research meetings that described by being informal to motivates each scientist to keep the adviser and all the group members updated on what everyone in the group is
doing. I presented my experimental results weekly, got the feedback and gained the ability to upgrade my laboratory skills.

**My learned clinical experience and skills:**

I joined clinical staff member whether consultants, registrars, clinical fellows and the Senior Specialist Nurse in Multidisciplinary Meetings (MDM) twice weekly, one event for clinical immunology cases and the other for allergic cases discussions. It was the time to share, to discuss and to recommend the best treatment modality based on UK and international guidelines bearing in mind, each individual patient’s circumstances and concerns. Clinical Immunology and Allergy Department at King’s College Hospital has 3 remarkable consultants, Dr Mohammad Ibrahim Consultant, Clinical Lead Honorary and Senior Lecturer, Dr Jimmy Hock Chye Gooi, Consultant Immunology and Dr Zoe Adhya, Consultant Allergy and Immunology; 2 registrars, Dr. Sameer Bahal and Dr. James Laffan; 2 clinical fellows, Dr. Ioasaf Karafotias MD, MSc and Dr. Helene Martini; and one Senior Specialist Nurse, Ms Theo Grosse-Kreul.

Clinical Immunology MDM held on Monday for discussing clinical immunological cases on the whole 3 hours (2pm-5pm). Every colleague presented about 3 -7 cases in each meeting whether he/she faced them in clinics, wards, or as a referral letter. In addition to commoner PID clinical cases scenarios, I learned clinical experience on how to approach a patient with recurrent infections efficiently, when to implement gene counseling in PID cases, why to monitor trough levels of Replacement immunoglobulin, who would we collaborate within other specialties to get best medical service and what are the clinical situations to ask for post vaccination immune response. I faced interesting cases like patients with Good’s syndrome (Thymoma with Immunodeficiency), DAVID syndrome, ADA 2 mutations, Phagocytic defects and secondary immunodeficiency.
Allergy MDM held on Thursday for discussing allergic cases at 10.30 Am. Every colleague presented 4 - 6 cases weekly. Variety of allergic cases were presented as Aspirin-Exacerbated Respiratory Disease (AERD), drug allergy, Hypocomplementemic Urticarial Vasculitis (HUV), Oral Allergy Syndrome, hereditary angioedema and I learned also more clinical experience in applying Evidence-based recommendations on Omalizumab for treating severe chronic spontaneous urticaria in people of 12 and over (applying NICE guidelines), offering Desensitization treatment for Allergic rhinitis and insect allergy and implementing different adjuvant aeroallergens control measures. Also I had opportunity to share my opinions in diagnostic approach of some patient and it was welcomed and discussed.

Also I had experienced MDM with teleconference as I attended this interesting MDM with respiratory and cardiovascular teams that supervised the same patient to take actions when situations applied to offer the best medical practice.

I get clinical attachment to the three brilliant consultants in the clinic times that are 4 times a week with average 100-150 patients monthly in each field allergic and immunological whether new, referral or fellow up patients. More medical knowledge and clinical skills I had learned from them like good doctor-patient relationship and clinical reasoning skills which are central to the healthcare practice.

Programmed investigation unit (PIU) is an inpatient unit where a full range of allergic investigational medicine and immunoglobulin replacement (IV IG) can be organized. I learned in vivo allergens testing, Drug provocation protocols, Vaccine injection in egg allergic patient with the help of Dr. Zoy Adhya interpretation and accompanying both friendly well trained registrars Sameer and James, where they offer answers to me when I want to clarify something and I was grateful to them. I joined Ms Theo Grosse-Kreul in IV IG preparations, immunotherapy and Omalizumab injections.
whether in PIU or Immunotherapy Clinic, I was grateful for her too much as I was exposed to International Nursing Group for Immunodeficiencies (INGID) review articles and European Nursing Guidelines for Immunoglobulin Administration.

Also, I get the opportunity to attend 2 Grand round meetings at King’s College Hospital that were held on Wednesdays, 1pm-2pm one was presented by respiratory team on “Bronchiectasis and immune deficiency” on 22 November and the other was presented by Gastroenterology team 20 September. Also, Dr. Mohammad Ibrahim invited 2 Professors to give a talk on genetic revolution in immunology. Fortunately, I was delighted to attend the meeting of Dr. Andrew Cant: President of European Society of Immunodeficiencies (ESID)

**What will I do after return to Egypt?**

As I stated my objectives in the motivation letter that I had sent for application to EAACI fellowship previously, I will adhere to my commitments. With building an allergy and clinical immunology center at Benha University Hospitals, hopefully, I can serve my community by presenting this medical service. Benha university hospital is located in the middle of Egypt delta and it is centered in Qualibya governorate inhabited by 4.9 million persons. I selected some of medical services that my medical community in genuine need to it aiming to settle more allergic and clinical immunology medical services after completion the medical team. In my first week after return to Egypt, I am working on report to present to my university administration to implement these medical services and I was delighted that university administration offers to help me in building this medical unit.

I am working to build collaboration with remarkable ENT, Respiratory specialists and laboratory immunologists to present comprehensive allergy and clinical immunology services for our community. Collaboration with allergists and immunologists in Egyptian Centers and exchange experiences is
fundamental to me. I am grateful to Dr. Mohammad Ibrahim when he offers to add help after my return in these objectives. He is fully eager to help, thanks too much to him.

I will continue doing laboratory experiments with conjunctions of local laboratory immunologists (I already build a link with Egyptian laboratory immunologists while I was in UK to implement my learned laboratory skills). I have some projects ideas to begin in Egypt and to share in many articles from allergic and clinical immunology perspective.

Aiming to attend next EAACI congress in 5/2018 to present our paper is one way to be involved more in EAACI activities that gave me this opportunity. In addition to that, I will focus on EAACI interest, work groups and task forces activities.

Also it is my pleasure to help EAACI junior members who propose to EAACI clinical fellowships by some advices if the EAACI jury doesn’t mind:

1. When choosing your scholarship destination, try using top ranking lists
2. Your scholarships begin 3-6 months earlier by your preparation work
3. Optimize your training for full benefit; one week for research and one for clinical stuff
4. Be adherent to your objectives that had been wrote in motivation letter
5. Don’t forget to taste the new city experience.

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Finally I would like to thank again EAACI jury, Clinical Immunology and Allergy Team at King's College hospital, London, UK. My acknowledgement towards their 3 remarkable consultants, Dr Mohammad Ibrahim Consultant, Clinical Lead Honorary and Senior Lecturer, Dr Jimmy Hock ChyeGooi, Consultant Immunology and Dr Zoe Adhya, Consultant Allergy and Immunology; 2 registrars, Dr. Sameer Bahal and Dr. James Laffan; 2 clinical fellows, Dr. Ioasaf Karafotias MD, MSc
and Dr. Helene Martini; one Senior Specialist Nurse, Ms Theo Grosse-Kreul, Scientific Research Officer, Dr Kruthika Sundaram, Clinical Scientist Mary Guckian, Biomedical Scientist, Terrence Hunter, finally to the most helpful person ever Dorota Parsons Department’s Medical Secretary.

My career is much progressed. In my culture, we have a saying, “the seekers of two concerns are never satisfied: one of them the seeker of knowledge ...” I think of myself simply as a seeker of knowledge!