RESEARCH ARTICLE

Deciphering the association of celiac disease and other comorbidities in patients with rheumatoid arthritis using systems and clinical medicine approaches

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Manuscript details:

Received: 31.03.2017
Accepted: 23.06.2017
Published :27.06.2017

Editor:
Dr. Arvind Chavhan

Cite this article as:
Sami Bahlas, Laila Abdullah Damiati, Nadia Dandachi, Hesham Sait, Peter Natesan Pushparaj (2017) Deciphering the association of celiac disease and other comorbidities in patients with rheumatoid arthritis using systems and clinical medicine approaches, *International J. of Life Sciences*, 5 (2): 141-150.

ABSTRACT

In this study, we decipher the association of celiac disease (CD) and other comorbidities in patients with rheumatoid arthritis (RA) using key clinical risk factors and systems medicine approaches for the differential diagnosis and personalized treatment.

In the systems medicine approach, we have used the Ingenuity Pathway Analysis knowledgebase (IPA, Qiagen, USA) to decipher the upstream regulators of the disease, canonical pathways, molecular networks and disease specific pathways commonly shared in RA and CD. Besides, eighty-two RA patients who have satisfied "American College of Rheumatology (ACR)" classification criteria for RA and 20 healthy volunteers were participated. RA patients with CD were identified based on serum levels of immunoglobulin A (IgA) autoantibodies to tissue transglutaminase (tTG-IgA). Besides, clinical parameters such as fasting blood sugar (FBS) and glycosylated hemoglobin $A1_C$ ($HbA1_C$) were measured to assess the diabetic state. Clinical parameters such as erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), C-reactive protein (CRP), mean cell volume (MCV), mutated citrullinated vimentin antibodies (anti-MCV), white blood count (WBC), albumin, calcium (Ca^{2+}) and vitamin D3 (VitD3) were analyzed in healthy volunteers, RA, and RA with CD cohorts respectively.

Systems medicine analyses showed that both RA and CD strongly associated with diabetes mellitus. Furthermore, clinical analyses indicate that FBS, $HbA1_C$, Anti-MCV, RF, CRP and tTG-IgA concentrations were significantly increased in both RA and RA with CD cohorts than healthy controls. tTG-IgA levels were significantly elevated in RA with CD cohorts compared with RA. On the other hand, anti-MCV levels were significantly increased in RA compared to RA with CD group. Besides, both RA and RA

with CD cohorts have significantly reduced levels of VitD3 and Albumin in the serum compared with healthy controls.

In conclusion, the RA and RA with CD cohorts have poor glycemic rheostat compared to healthy controls. Higher serum tTG-IgA levels in these cohorts indicate the susceptibility of RA patients to develop CD. The reduction in serum VitD3 levels in both RA and RA with CD cohorts further worsens disease prognosis. The systems medicine and clinical analyses, therefore, showed the potential association of diabetes mellitus (DM) and CD in RA patients.

Key words: Rheumatoid Arthritis, Diabetes Mellitus, Celiac Disease, Immunoglobulin A, Tissue Transglutaminase, Fasting Blood Sugar, Vitamin D3, Personalized Treatment.

Abbreviations: RA = rheumatoid arthritis, RF = rheumatoid factor, CD = celiac disease, FBS = fasting blood glucose, HbA_1C = glucosylated haemoglogin A_1C, CRP = C - reactive protein, DM = diabetes mellitus, tTG-IgA = tissue transglutaminase antibody, IPA = ingenuity pathway analysis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease of unknown etiology (Sangha, 2000), characterized by symmetric erosive synovitis, leading inevitably to the destruction of cartilage and bone as well as bursa and tendon sheaths of joints (Sangha, 2000; Smith and Haynes, 2002; Zeman and Scott, 2012; Okada *et al*., 2014; Biswas *et al*., 2011). The occurrence of RA in the general population is about 0.8-1% and the average age of onset is 40-60 years (Sangha, 2000; Smith and Haynes,2002; Zeman and Scott, 2012; Okada *et al*., 2014; Biswas *et al*., 2011; Lawrence *et al*., 2017). Conversely, RA may occur at any age in all the races and ethnic groups around the world. Intriguingly, there is a female preponderance of about 3:1, although the female to male ratio falls with increasing age to nearly 1:1 after 60 years of age. Patients with RA may have subtle onset of symmetric joint pain, swelling, that worsens during the course of time, associated with morning stiffness persisting more than 30 minutes and subsides during the day (Arnett *et al*., 1988). The severity of RA may range from mild to very intense; involving multiple organ systems leading to aggressive damage causing significant morbidity and mortality (McInnes and Schett, 2011). Although RA was once considered to be a relatively benign disorder, it is now known to be a disease with a strong tendency to shorten life and cause severe disability to varying degrees; accordingly RA is associated with a high social burden and economic cost due to unemployment (Hochberg *et al*., 1992).

Besides, recent studies have shown that RA patients also suffer from other autoimmune diseases like Celiac Disease (CD) (Meyer, 2004; Michelin *et al*., 2011; Evron *et al*., 1996). CD is a chronic autoimmune disease, of unknown etiology, caused by the digestion of gluten in genetically predisposed individuals leading to inflammation and damage in the lining of small intestine (Lauret and Rodrigo, 2013). In genetically predisposed individuals, eating food with gluten initiate an autoimmune reaction towards small intestine and subsequently causing the damage of villi. Additionally, the damage of the intestinal villi lead to reduced absorption of nutrients (Green and Cellier, 2007). Besides, there are many additional factors like family history with autoimmune diseases (AD) (Viljamaa *et al*., 2005; Neuhausen *et al*., 2008), genetic background in combination with epigenetic factors may also explain the reason for the development of CD (Gutierrez-Achury *et al*., 2011; Larizza *et al*., 2012).

Increased prevalence of CD has been documented in individuals with RA (Neuhausen *et al*., 2008) and juvenile inflammatory arthritis (Michelin *et al*., 2011; De Maddi *et al*., 2013). In the present study, we investigate the prevalence of DM and CD in RA patients, for better diagnosis and prognosis, using both systems medicine approach and clinical risk factors linked with these autoimmune diseases.

METHODS

Systems Medicine Analyses

Ingenuity Pathway Analysis (IPA) knowledgebase (Qiagen, USA) was used to query the number of genes implicated in the pathogenesis of CD (~ 55) and RA (~1105). These lists of genes, for both CD and RA, were subjected to core analyses to obtain the differentially regulated canonical pathways, upstream regulators of diseases, bio-functions and toxicological functions. Furthermore, the core analysis of CD and RA were compared using the comparison analysis module in IPA to understand the common canonical pathways, upstream regulators of diseases and bio-functions and toxicological functions and unique molecular networks in these diseases.

Patients

Eighty-two RA patients (4 males and 78 females) from Rheumatology Clinic at the King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia (KSA) who have satisfied "American College of Rheumatology (ACR)" classification criteria for RA were used in this study (Arnett *et al*., 1988). All the patients have provided written informed consent for their inclusion in this study and the anonymous use of their data from our "Rheumatoid Disease Registry", which has been approved by the KAUH ethical committee.

Clinical Analyses

C-reactive protein (CRP) was detected by latex agglutination slide test (Biocientifica, SA kit). The agglutination occurring within 2 minutes, show a CRP level equal or higher than 8 mg/L (Tillett and Francis, 1930). Cumulative clinical features were recorded for each patient during their last visit to the clinic. ESR was measured by Westergren method (Westergren, 1957). The WBC and MCV were estimated using automated cell counters (Shen and Blair, 2006; Vreugdenhil *et al*., 1990; Billett, 1990). In the serum, the mutated citrullinated vimentin antibodies (Anti-MCV) was measured by ELISA method (Gonzalez-Lopez *et al*., 2014; Renger *et al*., 2010; Bang *et al*., 2007), rheumatoid factor (RF) and albumin were estimated by Nephelometer (Orge *et al*., 2010; Mendler *et al*., 1999; Decavele *et al*., 2012). VitD3 was measured by electro-chemiluminescence method (Shakiba and Iranmanesh, 2013), fasting blood sugar (FBS) was estimated by hexokinase method (Xun *et al*., 2012; Swaminathan *et al*., 2013a; 2013b; Choudhry *et al*., 2014), glycosylated hemoglobin $A1_C$ ($HbA1_C$) was analyzed by Turbidimetry (Grey *et al*., 1996; Barrot *et al*., 2012), and calcium (Ca2+) was measured by Cresolphthalein method (Lorentz, 1982; Chapoteau *et al*., 1993; Kang *et al*., 2004). To identify CD in RA patients, we measured the levels of autoantibodies to tTG (Rashtak and Murray, 2007; van der *et al*., 2010).

Statistical Analyses

Statistical analyses were performed using SPSS Version 18.0 (SPSS Inc., Chicago IL, USA). The parametric data were expressed as Mean ± Standard Error (SE) and compared using Student's t-test (unpaired). Pearson correlation coefficient was also applied to find the correlation of clinical parameters observed among groups. *P*-values ≤ 0.05 were considered to be statistically significant.

RESULTS

The IPA analyses of the list of genes implicated in RA and CD showed that Tumor Necrosis Factor (TNF), Interferon Gamma (IFNG), Interleukin-6 (IL-6) and Interleukin-1 beta (IL-1b) are the common upstream regulators of the disease pathogenesis (Figure 1a).

Table 1. Demographic Characteristics of the Study Population.

	Control (n=20)	RA (n=72)	RA+CD (n=10)
Gender (Male/Female)	3/17	4/68	0/10
Age (years)	24.55 ± 2.91	44.67 ± 12.44	46.3 ± 14.05
Nationality (Saudi / Non- Saudi)	20/0	35/37	3/7

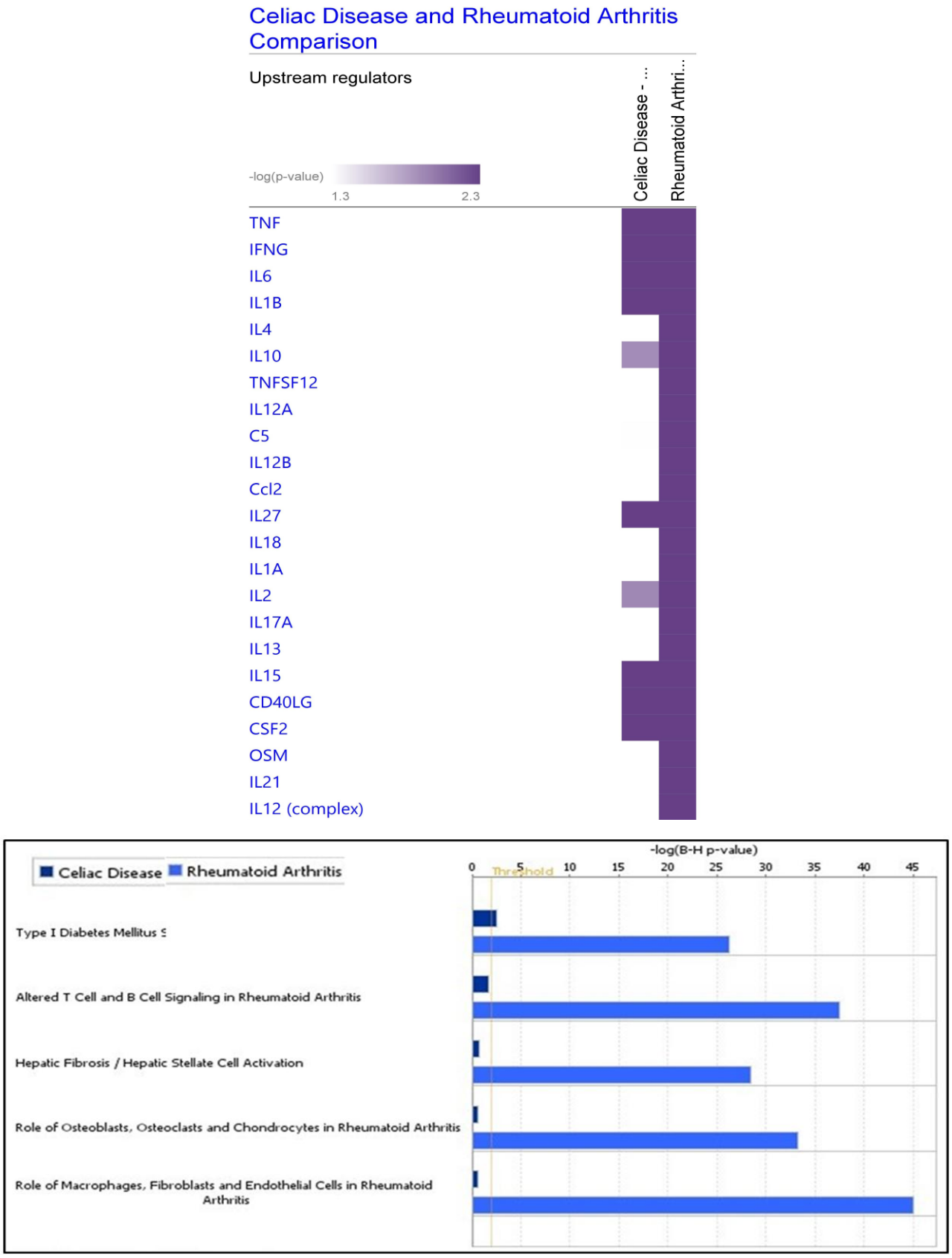

Figure 1. Systems medicine analyses of the differentially regulated genes in RA and CD using Ingenuity Pathway Analysis (IPA) Knowledgebase. (a) List of genes that are commonly upregulated in RA and CD. (b) The disease specific canonical pathways that are significantly upregulated in RA and CD. The top upstream regulators and canonical pathways were obtained using right tailed Fisher's Exact Test ($P<0.05$) and Benjamini-Hochberg (B-H) Multiple Testing Correction ($P<0.05$).

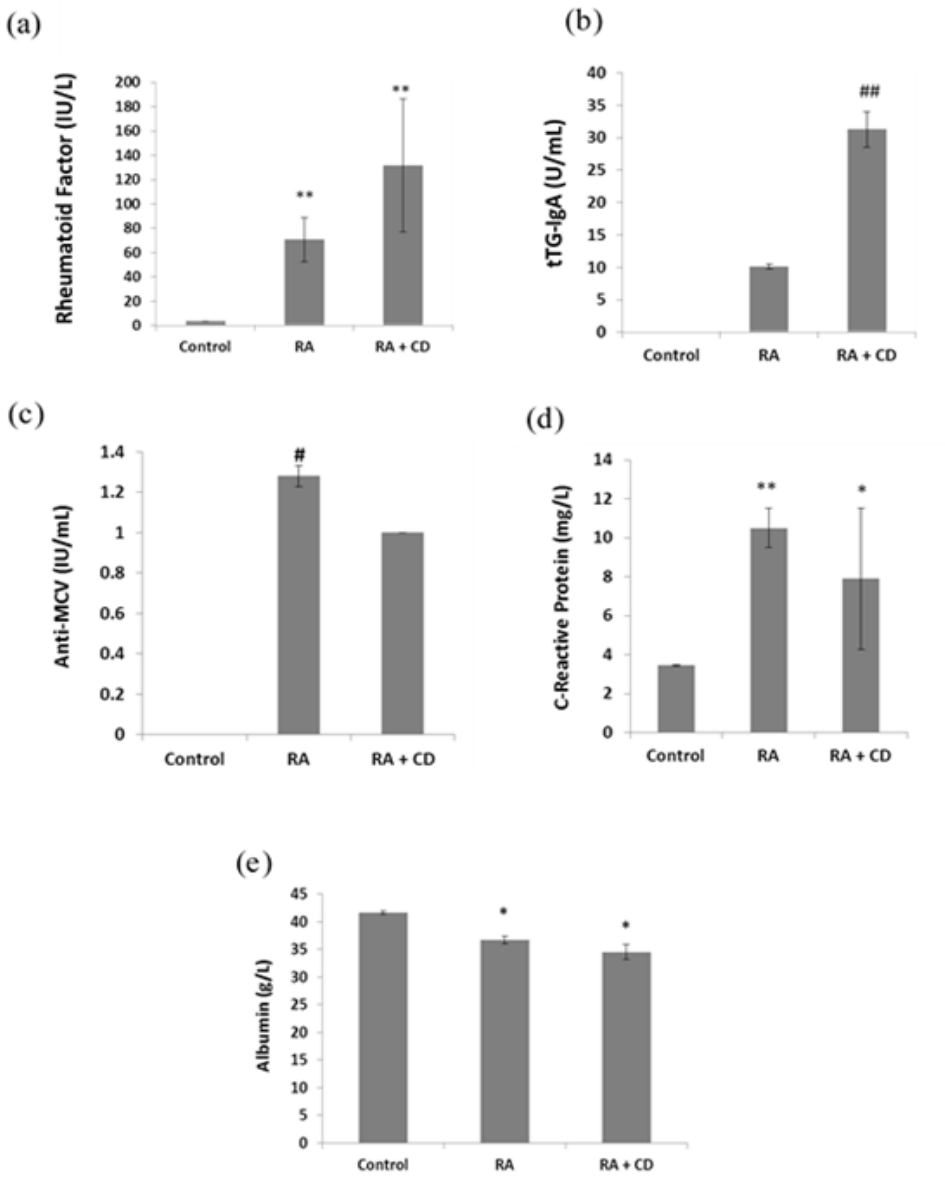

Figure 2. The serum concentrations of (a) RF (b) tTG-IgA (c) anti-MCV (d) CRP and (e) Albumin observed in Control, RA, and RA with CD cohorts. *P<0.05, **P<0.01 compared with control group; #P<0.05, ##P<0.01 comparison between RA and RA with CD cohorts.

Table 2. Clinical Characteristics of the Study Population.

	Group	Mean	Std Error	*P Value*
WBC (K/ µL)	*Control*	6.64	0.945521	0.864
	RA	6.69	0.30052	
	RA + CD	7.32	1.144745	
MCV (fL)	*Control*	-	0	0.962
	RA	79.76	1.335253	
	RA + CD	83.97	3.677729	
ESR (mm/H)	*Control*	11.8	1.397542	0.001***
	RA	30.49	1.058892	
	RA + CD	40.4	4.33232	
PLT (K/µL)	*Control*	268.4	7.940277	0.398
	RA	290.34	11.72029	
	RA + CD	252.23	38.40902	

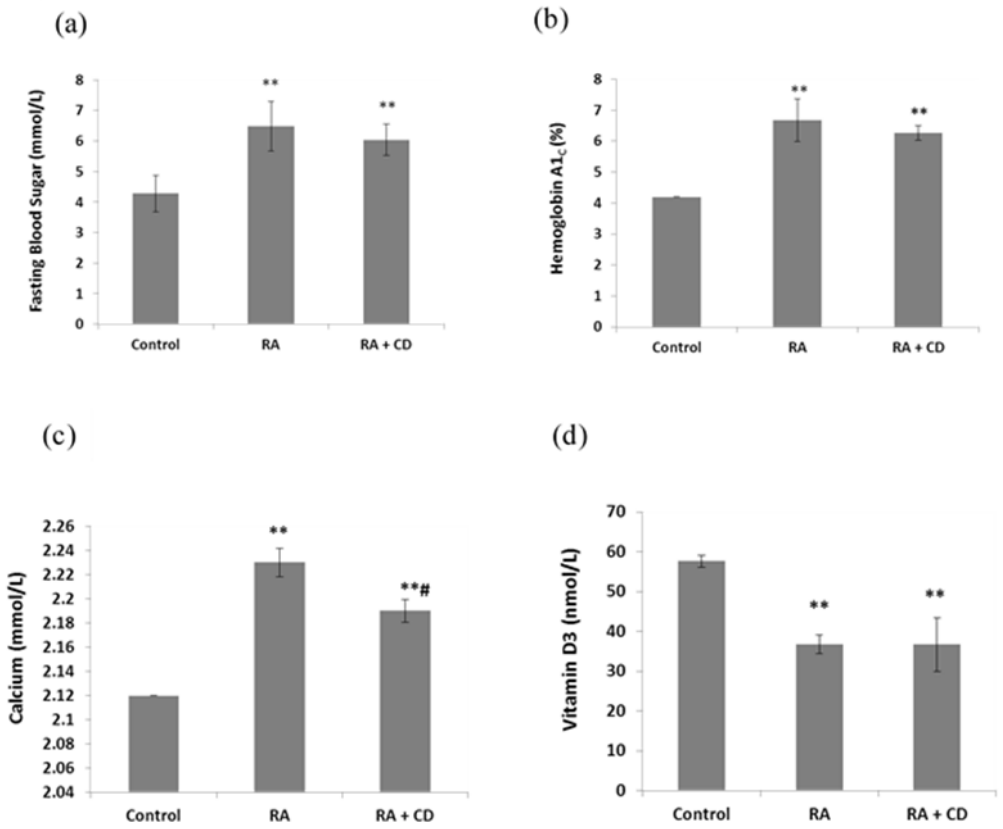

Figure 3. The serum concentrations of (a) The FBS (b) $HbA1_C$ (c) Calcium and (d) VitD3 observed in Control, RA, and RA with CD cohorts. *P<0.05, **P<0.01 compared with control group; #P<0.05, ##P<0.01 comparison between RA and RA with CD cohorts.

The clinical analyses was performed using blood samples obtained from 82 patients with RA. The mean age of RA without CD was 44.67±12.44 and RA with CD was 46.3 ± 14.05. The demographics characteristics of all the RA patients involved in this study were shown in Table 1. The observed levels of cellular parameters, such as WBC, MCV, ESR, and PLT in the blood of control, RA and RA with CD groups were summarized in Table 2. The ESR levels were significantly increased in RA and RA with CD groups when compared to control population (Table 2).

The RF, tTG-IgA, CRP, and anti-MCV levels were significantly increased in both RA and RA with CD groups compared to healthy controls (Figure 2). Besides, the RF was statistically increased in RA compared to RA with CD groups (Figure 2a). The IgA-TTG levels were significantly higher in RA with CD group when compared with RA group (Figure 2b). On the contrary, the anti-MCV levels were elevated in RA group when compared to RA with CD group (Figure 2c). The CRP levels were significantly elevated (Figure 2d) and the Albumin concentrations (Figure 2e) were significantly reduced in both disease groups.

The FBS and $HbA1_C$ levels were significantly increased in both RA and RA with CD groups compared to healthy controls (Figure 3a & Figure 3b). The calcium levels were significantly increased in both RA and RA with CD groups; however, statistically significant reduction was observed in RA with CD group compared to RA group (Figure 3c). On the other hand, the VitD3 levels were significantly decreased in both RA and RA with CD groups (Figure 3d).

DISCUSSION

Autoimmune diseases, such as, RA and CD have distinct clinical phenotypes (Mota *et al*., 1996), but the association of CD with RA was observed in genetically predisposed individuals and their family members

(Neuhausen *et al*., 2008; Zhernakova *et al*., 2011; 2013). The systems medicine approach specifically revealed that both CD and RA share an array of upstream regulators and other comorbidities like DM. Several studies show a common genetic basis for CD and RA as well as establish that 6 of the confirmed non-HLA RA-CD risk loci (out of 26 loci for each disease) are common (Zhernakova *et al*., 2013). A recent meta-analysis of data from genome wide association studies (GWAS) has identified eight additional RA-CD risk loci and single nucleotide polymorphisms (SNPs) that are implicated in antigen presentation and T-cell activation and could attribute to the disease pathogenesis Zhernakova *et al*., 2011). This could partly explain the association of CD in RA patients identified in our study cohorts. However, only 10 out of 82 RA patients (~ 8.2%) have developed CD with distinct disease pathogenesis as classified based on the clinical risk factors analyzed.

Studies have shown that both RF and anti-MCV were elevated in RA patients than CD patients (Renger *et al*., 2010). Besides, the increase in RF and anti-MCV levels in RA with CD cohorts was mainly due to RA than CD (Renger *et al*., 2010). On the other hand, tTG-IgA levels were increased in both RA and RA and CD cohorts. It indicates that RA patients may be predisposed to develop CD at some point in their life (Dzhambazov *et al*., 2009; Riente *et al*., 2004; Teichmann *et al*., 2010).

CRP levels were elevated in both RA and RA with CD patients. However, the levels may vary based on age and sex (Siemons *et al*., 2014). Besides, studies have shown that ESR and CRP values were positively correlated with each other, but both these factors may not be strongly associated with disease activity score (DAS) in these diseases (Siemons *et al*., 2014).

The significant increase in both FBS and HbA1c levels indicate a pre-diabetic state or/ an impaired glycemic control in RA and RA with CD patients that could be ascribed to the co-existence of CD with diabetes mellitus even though no potential link has, thus far, been established between CD and diabetes mellitus. However, a recent study has shown that CD is common in patients with diabetes mellitus (Hogg-Kollars *et al*., 2014). Also, studies have shown that insulin resistance and impaired beta-cell function are common in patients with RA (Ferraz-Amaro *et al*., 2013a; 2013b). Consequently, poor glycemic control was observed more in RA with CD compared to RA cohort.

Intestinal malabsorption in CD patients often results in vitamin and mineral deficiency (Hoffmanova and Andel, 2014). 1, 25-Dihydroxyvitamin D (1, 25 (OH) 2D3), otherwise called as VitD3, regulates both innate and adaptive arms of the immune system (Neve *et al*., 2014). The reduction in the VitD3 levels in both RA and RA with CD cohorts could be one of the reasons for the proinflammatory phenotype in these patients (Neve *et al*., 2014; Villanueva *et al*., 2012). Recent studies have documented the immunomodulatory and disease-modifying effects of VitD3 in various autoimmune diseases including RA and CD (Neve *et al*., 2014; Villanueva *et al*., 2012; Dehghan *et al*., 2014; Hansen *et al*., 2014; Tavakkoli *et al*., 2013). Supplementation of VitD3 in immune cells, such as monocyte derived macrophages, from RA patients reduced the Nitric Oxide (NO) levels and other proinflammatory mediators such as IL-1α, IL-1β, IL-6 and RANKL (Neve *et al*., 2014). Likewise, the higher frequencies of osteoporosis, osteopenia and low bone mineral density (BMD) in RA and CD were attributed to the low VitD3 levels (Chen *et al*., 2014; ojeda-Rivera *et al*., 2012; Kemppainen *et al*., 2012). However, intake of gluten-free diet and VitD3 supplementation might restore VitD3 levels in RA with CD patients (Molteni *et al*., 1995; Hallert *et al*., 2002; Duerksen *et al*., 2012).

We have observed a reduction in the levels of serum albumin in both RA and RA with CD groups. Previous studies have also reported lower levels of serum albumin in RA patients (Ahlstrom *et al*., 1956; Shetlar *et al*., 1959). The reduction in serum albumin in RA and RA with CD cohorts may be attributed to the malabsorption of dietary albumin in the intestine. Albumin helps to clear calcium from the blood and the reduced albumin levels in RA and RA with CD cohorts might increase the calcium levels in the serum. However, temporary hypocalcemia and hypercalcemia were commonly observed in RA patients, though most of the time calcium levels were normal (Keshgegian *et al*., 1994). It was also observed that changes in calcium levels were not correlated with clinical, hematological, or immunological parameters of disease activity (Keshgegian *et al*., 1994). However, in CD patients, the hypocalcemia may be prevalent due to malabsorption of calcium in the intestine (Molteni *et al*., 1995).

The peripheral blood mononuclear cells (PBMC) from RA patients exhibited increased basal Ca (2+) concentrations with a significantly reduced capacity to respond upon acetylcholine (Ach) stimulation compared to healthy controls. This phenomenon

contributes partially to the disturbed neuroimmune interaction in RA patients (Nast *et al*., 2009). The calcium-sensing receptor (CaSR) expression was increased in the circulating monocytes of RA patients with severe coronary artery calcification (Paccou *et al*., 2014). A recent study shows that Calcium Gluconate administration improves collagen-induced arthritis (CIA) in mice (Orge *et al*., 2010). Calcium and VitD3 (CaD) supplementation in women improves RA disease activity and CaD reduced loss of BMD in the lumbar spine and trochanter in RA patients treated with low-dose corticosteroids (Furuya, 2011).

In conclusion, our study indicates that RA and RA with CD cohorts have poor glycemic control compared to healthy controls. This warrants proper screening and control of FBS in both RA and RA with CD patients. Elevated serum tTG-IgA levels in both RA and RA with CD cohorts indicate the susceptibility of RA patients to develop CD. The reduced serum VitD3 levels in both RA and RA with CD cohorts further worsens disease prognosis. However, strict adherence with gluten-free diet as well as VitD3 supplementation might reduce the complications of CD in RA patients. Our study, thus, provide cue(s) for the differential diagnosis of RA patients with CD and guides in formulating personalized treatment programs for efficient therapeutic outcomes in these patients.

Acknowledgements

Prof.Sami Bahlas is currently supported by DSR small grants. Dr.Peter Natesan Pushparaj is funded by the King Abdulaziz City of Science and Technology (KACST) Strategic Grants (KACST 12-BIO2719-03 & 12-BIO2267-03). We thank all the patients and healthy volunteers for their participation in this study.

Conflicts of interest: The authors, hereby, declare that they have no conflict of interest related to this work

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