IRA **E-BULLETTIN**

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WINNER'S COLUMN

ORAL BASIC BEST PAPER

We have studied the effect of curcumin on cytokines of T-helper subsets and innate immune cells in minor salivary gland (MSG) tissues of patients with primary Sjogren's syndrome (pSS) by in-vitro culture experiment. Results of the study showed curcumin significantly decreases levels of mRNA expression and protein levels of Interleukin-6 (IL-6) as well as IL-1β in MSG tissues of pSS compared to SICCA controls. Treatment with curcumin reduces levels of pro-inflammatory cytokines in MSG tissues of pSS.

Title: Effect of curcumin on pro-inflammatory cytokines in primary Sjögren's syndrome

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Abstract:

Introduction: Curcumin reduces disease severity and ameliorates lupus-like/ Sjögren's Syndrome-like disease in mice model. The immunological basis of these effects is unknown. This study examined the effect of curcumin on pro-inflammatory cytokines secreted by salivary glands in patients with primary Sjögren's syndrome (pSS).

Methods: Minor salivary gland (MSG) tissue was collected after obtaining written consent from patients undergoing biopsy as a part of evaluation for suspected pSS. The tissue was treated with phytohemagglutinin (PHA) alone as well as PHA with curcumin (30 μM) and cultured in RPMI 1640 medium for 48 hours at 37°C in a CO2 incubator. After the incubation period, culture supernatant and tissue were stored in the ultra-deep freezer (-80°C). IL-6 levels were measured in supernatant by commercially available ELISA kits. RNA was extracted from tissues using the Tri reagent method. Expression of pro-inflammatory cytokines, IL-6, IL-8, TNF-α, IL-1β, IL-4, IL-21, IL10 and IFN-γ was done by qPCR. Differences between groups were tested by Student's t-test.

Results: Forty-seven patients were recruited. Eight patients satisfied ACR/EULAR criteria for pSS. Seven patients with absent glandular inflammation and negative serologies constituted controls. In the pSS group, but not in controls, median IL-6 levels in supernatant were less in the curcumin-treated group as compared to PHA alone (5.5 (0.7–1.3) vs. 18.3 (12–32) ng/mL; p=0.04). mRNA expression levels of IL-6 and IL-1β were lower in curcumin-treated groups as compared to PHA alone in both cases and controls (p=0.0009 & p=0.04, respectively). There was no difference in other cytokine levels between the treatment groups. mRNA expression levels of IL-4, IL-21, and IL10 were below detectable range.

Conclusion: Curcumin reduces secretion of IL-6 levels in salivary gland tissues of patients with pSS. Curcumin suppressed PHA-induced mRNA expression levels of IL-6 and IL-1β in MSG tissue of pSS and sicca controls.

ORAL CLINICAL BEST PAPER

Our work focused on cardiovascular risk in rheumatoid arthritis. We always felt the inadequacies in cardiovascular risk screening and preventive strategies in our patients with rheumatic disease. However, no formal study was done to assess the quantum of the problem. “We need to state out the problem before we set out to do anything further…” was what Prof. Vikas Agarwal told me when this project was conceptualized. We estimated the prevalence of traditional cardiovascular risk factors in our rheumatoid arthritis population. One-fourth of our patients should have been on at least moderate-intensity statin therapy according to AHA guidelines if formal CV screening had been in our patients, while none of them were. Despite a mean age of 47 years, 27% of our patients had subclinical atherosclerosis as well. We also observed the variability between various risk-predicting algorithms and felt the inadequacies of using them in the Indian population. Our study calls for studies validating CV risk algorithms in our population. More importantly, it is an eye-opener reflecting the sub-optimal comorbidity management in rheumatology clinics.

Title: Cardiovascular Risk Assessment in Indian Rheumatoid Arthritis Patients: Comparison of Risk Algorithms and Carotid Intima-Media Thickness

Authors: Hafis Muhammed, Durga P Misra, Sujata Ganguly, Sarit Sekhar Pattanaik, Saurabh Chaturvedi, Harshit Singh, Mohit K Rai, Anamika Anuja, Namita Mohindra1, Neeraj Jain1, Sudeep Kumar2, Vikas Agarwal

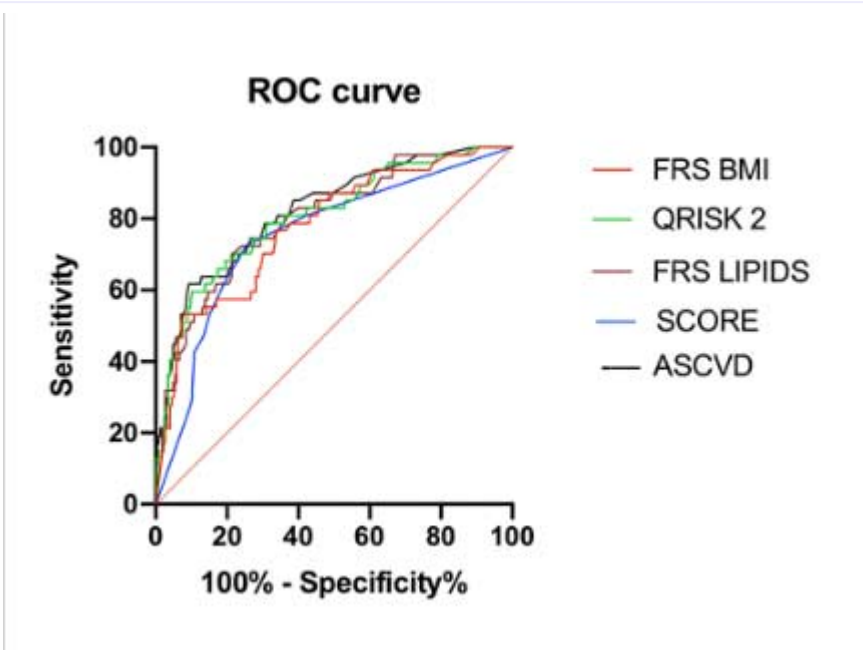
Affiliation: Department of Clinical Immunology and Rheumatology, 1Department of Radiodiagnosis and 2Department of Cardiology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India

Abstract:

Background: Rheumatoid arthritis(RA) patients have increased cardiovascular(CV) risk with no data on CV risk scores in Indian patients. We aimed at studying CV risk in RA patients.

Methods: Patients fulfilling 2010 ACR/EULAR criteria for RA were included. Presence of CV risk factors were recorded. Ten-year–CV risk was predicted using Framingham Risk scoring using lipids(FRS-Lipids), Framingham Risk scoring using body mass index (FRS- BMI), QRISK-2, SCORE and the algorithm recommended by ACC/AHA (ASCVD) in patient who were 40 years or older. Carotid Intima Media Thickness(CIMT) was measured on far-wall of the common carotid artery. Subclinical atherosclerosis was defined as CIMT >0.90 mm or presence of plaque.

Results: Three hundred and thirty-four patients (M: F = 49:285, Mean age =47.16±12.57 years) were enrolled; 6% were smokers, 12% had diabetes mellitus (DM), and 21% had hypertension. Mean CIMT was 0.70±0.15 mm. In univariate analysis, mean CIMT significantly differed according to gender and presence or absence of erosions, extra articular manifestation (EAM), DM, and hypertension. CIMT correlated significantly with age, disease duration, systolic blood pressure, and total cholesterol. Multiple regression analysis showed age, EAM, and male gender as independent predictors of CIMT (r2=0.431 for final regression model). All risk scores had moderate correlation with CIMT with maximum for QRISK (r=0.570). Percentage of patients with predicted >10% risk varied from17.6% to 41.9% between scores. Agreement between scores in predicting risk was moderate in general with maximum agreement between QRISK2 and FRS-Lipids (weighted kappa:0.790). ASCVD and QRISK-2 showed maximum sensitivity when subclinical atherosclerosis assessed by CIMT was taken as standard (AUC: 0.822 and 0.806 respectively) as shown in below figure.



Conclusions: Prevalence of risk factors and subclinical atherosclerosis were calculated. Age, EAM, and male gender independently predicted CIMT. While further validation is required in terms of clinical end points, our findings suggest that risk algorithms cannot be used indiscriminately in RA.

BEST POSTER BASIC

Vallayyachari Kommoju

The present study was designed to understand the influence of synovial inflammatory milieu on peripheral blood Treg cells in RA patients. Phenotypic characterization, gene expression profiling, and cytokine concentrations were analyzed by FACS, RTPCR, and CBA methods, respectively. Results showed Tregs in RA are converted to Th1 & Th17 phenotype on exposure to inflammatory cytokine milieu in the synovial fluid, thus losing their regulatory functions. Understanding factors influencing the stability of Treg cells may help improve future therapeutics.

Title: In vitro characterization of Treg cells isolated from peripheral blood of Rheumatoid Arthritis patients.

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Abstract:

Background: Present study was designed to understand the influence of synovial inflammatory milieu on peripheral blood Treg cells in patients with Rheumatoid Arthritis (RA).

Methods: The Peripheral Blood (PB) and synovial fluid (SF) of RA (n=80) and OA (n=30) patients were analyzed for CD4+T-cell subset frequencies and phenotypes by flow cytometry. Cytokine concentrations in plasma and SF were measured by cytometric bead array. Tregs from 5 RA-PB were isolated and cultured in autologous synovial fluid for 24 h. Phenotypic expression of Th1 and Th17 chemokines on the cell surface were analyzed by flow cytometry and expression levels of T-bet, RORγ, and FOXP3 in the cultured Treg cells were measured with quantitative real-time PCR (RT-qPCR).

Results: The PB and SF frequencies of Th1, Th17, and Tregs are shown in Table 1. The pro-inflammatory cytokines were high in the plasma and SF of RA but the anti-inflammatory cytokines were similar (Fig1.A&B). Treg cells isolated from PB and cultured in autologous SF of RA showed increased cell surface expression of CXCR3+ and CCR6+ (Fig1C). Gene expression studies showed an increased expression of T-bet, RORγ and decreased expression of Foxp3 after in vitro culture (Fig1D).

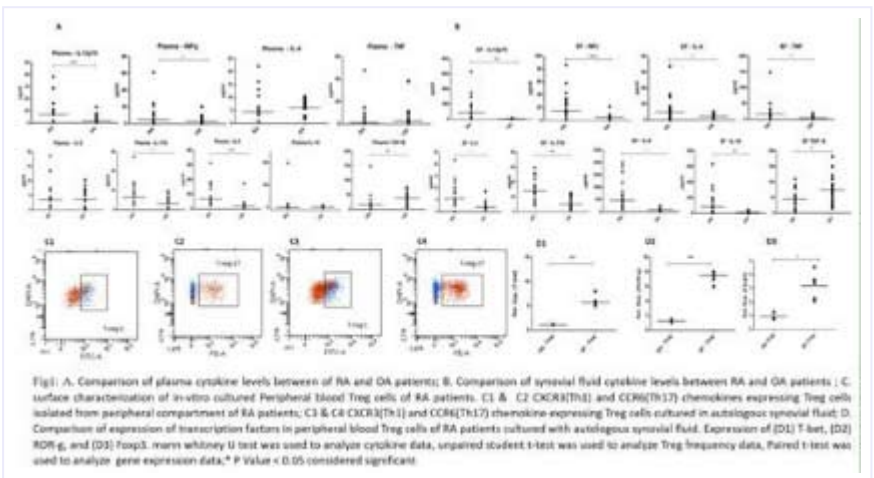

Fig1: A. Comparison of plasma cytokine levels between of RA and OA patients; B. Comparison of synovial fluid cytokine levels between RA and OA patients ; C. surface characterization of in-vitro cultured Peripheral blood Treg cells of RA patients. C1 & C2 CXCR3(Th1) and CCR6(Th17) chemokines expressing Treg cells isolated from peripheral compartment of RA patients; C3 & C4 CXCR3(Th1) and CCR6(Th17) chemokine-expressing Treg cells cultured in autologous synovial fluid; D. Comparison of expression of transcription factors in peripheral blood Treg cells of RA patients cultured with autologous synovial fluid. Expression of (D1) T-bet, (D2) RORγ, and (D3) Foxp3. mann whitney U test was used to analyze cytokine data, unpaired student t-test was used to analyze Treg frequency data, Paired t-test was used to analyze gene expression data;* P Value < 0.05 considered significant

Conclusion: Tregs in RA are converted to Th17 phenotype on exposure to inflammatory cytokine in the synovial fluid, thus losing their regulatory functions. Understanding factors influencing stability of Treg cells may help improve future therapeutics.

BEST POSTER CLINICAL

Adult-onset hypophosphatemic osteomalacia can be a close mimic of rheumatological condition, specially spondyloarthritis (both clinically and radiologically).

-Serum calcium and phosphorus level measurement should be done for all vague aches and pains not responding to analgesics.

-FGF23 level should be checked for hypophosphatemia resistant to correction.

-DOTANOC PET CT is a better modality than FDG PET CT for the detection of oncogenic osteomalacia.

-Tumor responsible for oncogenic osteomalacia is a slow-growing benign tumor and removal of the tumor leads to the correction in phosphorus level and symptoms.

Title: Hypophosphatemic Osteomalacia – a close mimic of rheumatological disorders

Authors: Ishita Shah, Vaibhavi Velangi, Yogesh Preet Singh, Abhishek Patil, Sharath K, Vikram Jain, Aditya Hegde, Karthik Prabhakar, Vishad Vishwanath, Pooja Belani

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Abstract:

Background: Hypophosphatemia due to increased urinary phosphate excretion is the predominant cause of osteomalacia seen with the disorders of vitamin D metabolism. Hypophosphatemic osteomalacia can occur due to hereditary hypophosphatemic rickets syndrome or tumor-induced osteomalacia. Drug-induced Fanconi syndrome can result in renal phosphate wasting and osteomalacia. Here we present 12 cases of hypophosphatemic osteomalacia with different etiologies most of whom were referred to a rheumatologist with a diagnosis of spondyloarthritis.

Method: Data was collected retrospectively from medical records from January 2015 to September 2019. Osteomalacia was diagnosed by Bingham and Fitzpatrick criteria (two of the following: low calcium, low phosphate, elevated alkaline phosphatase or suggestive radiographs).

Discussion: Adult-onset osteomalacia can be overlooked due to the lack of specific clinical features. Axial involvement in osteomalacia is commonly confused with spondyloarthropathy due to overlapping clinical features like back pain and radiological features like fuzzy sacroiliac joint outlines, calcified enthesopathies and subchondral bone resorption. Elevated serum FGF23 level and increased phosphate excretion in urine with tubular maximum phosphate reabsorption per glomerular filtration rate (TMP/GFR) reduction help to confirm the diagnosis of hypophosphatemic osteomalacia. Tumors responsible for oncogenic osteomalacia are slow-growing benign tumours and removal of tumour leads to immediate and permanent improvement in the serum phosphate level.

Conclusion: Adult-onset hypophosphatemic osteomalacia can be a close mimic of rheumatological condition, specially spondyloarthritis (both clinically and radiologically). Serum calcium and phosphorus level measurement should be part of evaluation of vague aches and pains not responding to analgesics. In patients not improving to phosphate correction FGF23 level should be checked. DOTANOC PET- CT should be performed to look for oncogenic osteomalacia. DOTANOC PET–CT is a better modality for tumor detection as compared to FDG PET–CT. Resection of tumor helps considerably to alleviate the patient's condition.

BEST POSTER CASE

This patient intrigued me in more ways than one. It took two years to pinpoint the diagnosis and the treatment after establishing the diagnosis was an even bigger challenge. With support from various quarters we've been able to give him tocilizumab for the past eight months. I'm hoping I can raise funds for his treatment.

Abstract:

Title: H syndrome: a novel genodermatosis mimicking IgG4 related disease

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Case: A 24-year–old male was symptomatic for the past five years with abdominal pain and loose stools alternating with constipation. He noticed thickening and darkening of skin on both his lower limbs and dryness of mouth and eyes with caries of multiple teeth. Hyperpigmentation and induration were noted on both lower limbs extending till lower abdomen prominent on inner thighs and sparing both the knees. (Figure 1) A firm mass was noted in the pelvic region. .Colonoscopy revealed superficial ulcers with mild luminal narrowing in the distal ascending colon with dense inflammatory infiltrate on biopsy. PET CT revealed FDG

avid multiple parenchymal opacities in both upper lobes and sheet-like soft tissue thickening with mild FDG uptake involving thoracic aorta, retroperitoneum, and pelvic fascia with skin and subcutaneous involvement in inguinal and thigh regions. FDG uptake was also noted in the hepatic flexure with thickening of the wall. Double inferior vena cava was seen. Biopsy of pelvic mass revealed extensive fibrosis. Immunostaining for IgG4 was negative. IgG4 levels were within normal limits. A diagnosis of H syndrome was considered in view of characteristic cutaneous findings. Clinical exome sequencing revealed a previously reported pathogenic variant c.1330G>T [p. E444Ter; HGMD ID- CM093097] in homozygous state in SLC29A3 confirming the clinical diagnosis of H syndrome. He was started on tocilizumab with symptomatic response.

Discussion: H syndrome is a rare autosomal recessive disease characterized by cutaneous hyperpigmentation, induration, and hypertrichosis along with numerous systemic manifestations .The clinical manifestations are heterogenous and include short stature, diabetes mellitus, hypogonadism, flexion contractures, and heart anomalies. Only ten cases have been reported from India.

Conclusion: Tocilizumab may be an effective treatment for H syndrome.

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