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STUDY OF ASSOCIATION OF INTESTINAL PREVOTELLA IN EARLY RHEUMATOID ARTHRITIS

General Medicine

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ABSTRACT

BACKGROUND: - Despite being highly prevalent and highly studied autoimmune disease, the etiology for the autoimmunity in Rheumatoid arthritis is not known. One of the recent advances being the role of gut microbiota.

AIMS AND OBJECTIVES:- To study the association of Prevotella copri in the gut microbiota in untreated Rheumatoid Arthritis patients

MATERIAL AND METHODS: Stool samples were collected from controls and new onset, untreated Rheumatoid arthritis patients in reduced transport fluid (RTF) and were subjected to anaerobic culture in Kanamycin-Vancomycin blood agar to identify Prevotella species based on colony morphology and biochemical tests. Inoculated plates were incubated anaerobically by gas pak method for 72 hours. Also, broadband PCR was run on stool samples collected in RTF for detection of 16S RNA of Prevotella species and the samples which test positive were further subjected to specific PCR with another set of internal primers to detect 16S RNA of Prevotella copri. HiPurATM Stool DNA Purification Kit (#MB544 HIMEDIA) was used for extraction of bacterial genome. Separation of genomic DNA was done using agarose gel electrophoresis. 2 sets of primers were used among which one is universal primer for bacterial species i.e., 16S rDNA to validate the sample or DNA for bacterial genomic study. Another primer mix specific to Prevotella copri was procured from Helini Biosciences along with positive control for Prevotella copri to identify samples positive for Prevotella copri.

RESULTS:- Stool samples were collected from 30 cases and 25 healthy controls and were subjected to PCR and culture. Anaerobic culture showed no growth of Prevotella. PCR studies showed 19(63%) cases being positive for Prevotella copri nucleic acid whereas only 7(28%) samples were positive in controls.

CONCLUSION: - Intestinal Prevotella copri was found in significant number of cases of Rheumatoid arthritis compared to controls (p value: 0.009) indicating the alteration in gut microbiota in cases, which could be the reason for priming of autoimmunity in Rheumatoid arthritis.

KEYWORDS

Rheumatoid Arthritis, Intestinal Prevotella Copri, Gut Microbiota, Pcr And Culture

INTRODUCTION:-

Rheumatoid arthritis is a highly prevalent systemic autoimmune disease with predilection for the joints. If left untreated, RA can lead to chronic joint deformity, disability, and increased mortality. Despite recent advances towards understanding its pathogenesis, the etiology of RA remains elusive. Many genetic susceptibility risk alleles have been discovered and validated but are insufficient to explain disease incidence. RA is therefore a complex disease requiring both environmental and genetic factors for onset.

Among environmental factors, the intestinal microbiota has emerged as a possible candidate responsible for the priming of aberrant systemic immunity in RA. Recent studies demonstrate that gut microbiota has a much more far-reaching influence on the immune system, beyond the gastrointestinal tract and may lead to autoimmune and allergic diseases both within and outside the gut apart from gut associated diseases like Crohn's and other inflammatory bowel diseases[1,2]

Therefore demonstration of definite role of gut microbiota in RA and understanding of how we can restore the dysbiosis in the gut might represent a new dimension in immunology research and therapy and in the pathogenesis and treatment of RA. The human gut microbiota has been classified into unique enterotypes, one of which is defined by the predominance of Prevotella species, and in previous studies, Prevotella copri has been shown to be associated with untreated RA patients.

AIMS AND OBJECTIVES

To study the association of Prevotella copri in the gut microbiota in untreated Rheumatoid Arthritis patients.

MATERIALS AND METHODS

Out-patient and In-patient subjects with clinical diagnosis of early untreated RA attending JSS Hospital during the study period.

SAMPLE SIZE

30 cases of early Rheumatoid arthritis and 25 Healthy controls.

METHOD OF COLLECTION OF DATA

TYPE OF STUDY: Comparative Cross-sectional Study.
STUDY PERIOD: 18months

INCLUSION CRITERIA:

New onset RA with a duration of minimum of 6 weeks and upto 6 months from diagnosis and Patients who are not-treated with DMARDS, biologic therapy or steroids were included.

EXCLUSION CRITERIA

Recently on any antibiotic therapy (<3 months) and patients on Total parenteral nutrition.

Known Inflammatory bowel disease or known history of malignancy.

Current consumption of pre/probiotics.

Any gastrointestinal tract surgery leaving permanent residue (gastrectomy,bariatric surgery, colectomy), or significant liver, renal or peptic ulcer disease

RESULTS

In the study population 5(17%) patients were in the age group 18 - 30 , 12(40%) between 31- 40 , 7(23.3%) in the age group 41 – 50, 5(16.7%)

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between 51 – 60 and 1(3.3%) above 60 years age. Maximum number of patients were from the age group of 31 – 40. Out of 30 patients 25(83%) were females and 5(17) were males.

RF test and Anti ccp ELISA were also done for all cases of RA. In the study population 17(57%) patients were positive and 13(43%) were negative for RF, 7(23%) were positive and 23(77%) were found to be negative for Anti ccp ELISA

Anaerobic culture of stool samples were negative for P.copri both in RA and healthy controls.Polymerase chain reaction was carried out for all 30 NORA cases and 25 healthy controls. In NORA samples 19 (63%) were positive and 11(37%) were found to be negative and in 25 healthy controls 7(28%) were positive and 18(72%) were negative.

Table 1. PCR Results in cases and controls

Samples	Number	Percentage
Positive RA samples	19	63%
Negative RA samples	11	37%
TOTAL	30	100%
Positive control samples	7	28%
Negative control samples	18	72%
TOTAL	25	100%

Graph 1. Comparison among PCR results among RA cases and Healthy controls

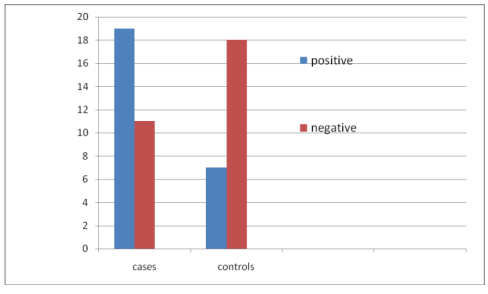

Table 2.Chi-square test for significance of PCR results

	Value	df	Asymp. Sig. (2- sided)
Pearson Chi-Square	6.830	1	0.009

DISCUSSION

Rheumatoid Arthritis (RA) is a chronic debilitating disease known from ancient times. It is a disease of disability, deformity and death due to increased cardiovascular risk. RA is a result of the complex play of various genetic and environmental factors that lead to the

ignition and perpetuation of the disease. Over years, there has been enormous research in the field of RA to find out the etiopathgenesis of RA. Though it is well known that autoimmunity is the final culprit responsible for joint destruction and various systemic manifestations of RA, the trigger for the autoimmunity is the question unanswered till date. Among environmental factors, the intestinal microbiota has emerged as a possible candidate responsible for the priming of aberrant systemic immunity in RA[3]. A marked association with anaerobic bacteria, especially the Prevotella species predominantly P.copri has been observed in many studies(Jose U Scher et al 2013)[4].

P.copri was detected by PCR in 19 cases out of the total study population of 30 cases (63%). Only 7 out of the 25 healthy controls tested positive for P.copri DNA by PCR (28%). In a study conducted by Jose U Scher et al., 75% of NORA patients and 21.4% of healthy controls carried P.copri in the intestinal microbiota(3).

Maeda et al noticed increased Prevotella copri in 1/3 of RA patients (33.3%) and decreased Bacteroides in 1/3 of RA patients(4). Zhang et al found there was increased abundance of P. copri with duration of RA in the first year(5).

Pianta et al in their study found, Th1 responses to Pc-p27 protein of P.copri in 42% of NORA patients. They also found IgG or IgA antibody responses to Pc-p27 proteint in 22% of NORA and 33% of chronic RA patients Most importantly, of 18 patients in whom both serum and synovial fluid were available, 3 of 5 patients with IgG P.copri antibodies had 16S DNA for Prevotella detected in the synovial fluid. P.copri was also detected in normal subjects in different studies but to a lesser extent compared to RA patients(6).

In the present study, out of 25 healthy controls,7(28%) had P.copri in the gut microbiota which is comparable to study done by Jose U Scher et al., 21.4% of healthy controls carried P.copri in the intestinal microbiota. Recently study conducted by Alpizar Rodriguez D included 133 participants of which 50 were classified as first degree relatives controls and remaining as pre clinical RA, concluded prevotella spp enrichment in individual with preclinical RA (7)

Table 3. Comparison of results of different studies on association of RA and P. copri

Study	Results
Scher et al	Increase in P. copri was noted in 75% of untreated cases compared to 21.4% in healthy controls
Maeda et al	Increased P. copri in 1/3 of RA patients and decreased Bacteroides in 1/3 of RA patients. Early RA patients showed dominance of P. copri in the gut Microbiota
Zhang et al	Increased abundance of P. copri was found during the first year as the duration of RA increases
Pianta et al	Prevotella copri 16S DNA detected in 3 out of 5 patients with IgG antibody response to P.copri. Increased Th1 responses to Pc-p27 protein of Prevotella copri found in 42% of NORA patients
Alpizar Rodriguez D	Increase incidence of prevotella sps in preclinical RA compared to first degree relatives controls
Current study	63% cases were positive for P. copri compared to 28% in healthy controls

CONCLUSION

There was a significant association of Prevotella copri with new onset Rheumatoid Arthritis as compared to healthy adults in the current study.

1. The organisms role may be triggering the immunopathogenesis of RA, the aetiology of which is due to environmental and genetic factors combined together.
2. Anaerobic culture was not sensitive to grow the organism in vitro. This could be due to overgrowth of commensal flora or few organisms in the gut flora.
3. Prevotella copri nucleic acid could be detected in a significant number of RA samples by PCR as compared to healthy controls (p value = 0.009).
4. The study needs to be extended to larger number of samples and to be correlated with culture in order to establish a more definitive role of Prevotella species in the aetiopathogenesis of Rheumatoid arthritis.

RECOMMENDATION:-

we strongly recommend to screen patients of newly detected Rheumatoid arthritis for Prevotella copri.

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