International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 01-03-2019; Accepted: 03-04-2019
www.medicalsciencejournal.com
Volume 5; Issue 5; May 2019; Page No. 06-08

Prevotella copri sp. isolated from human faeces associated with new onset rheumatoid arthritis (NORA)

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Abstract
Prevotella species are present in humans, often existing in the oral cavity, gastrointestinal tract, and vagina. In humans, they can act as an opportunistic pathogen. The present study entitled "Study of association of intestinal prevotella in new onset Rheumatoid Arthritis (NORA)" was carried out in Department of Microbiology, JSS Hospital from March 2016- March 2017. A total of 30 stool samples for NORA cases and 25 for healthy controls were included in this study. In NORA cases maximum number of patients were from the age group of 31-40. Samples from NORA cases were screened for RF and Anti CCP testing by ELISA. RF showed 17 positive cases whereas Anti CCP were positive for 7 cases. Anaerobic culture of stool samples was negative for *P. copri* in both NORA and Healthy controls.

Keywords: *Prevotella copri*, faeces, rheumatoid arthritis, NORA

1. Introduction
Rheumatoid arthritis (RA) is a highly prevalent systemic autoimmune disease with predilection to involvement of joints. If left untreated, RA can lead to chronic joint deformity, disability & increased mortality. RA is caused by combination of genetic & environmental factors. RA can be specifically triggered or exacerbated by a number of infections or by external chemical or physical agents, including tobacco smoking & silica among others [2]. The role of intestinal microbiota in the pathogenesis of RA possibility for the priming of aberrant systemic immunity [1]. Mucosal dysbiosis has long been suggested as playing a role in the development of RA & numerous microorganisms resident to a variety of host mucosal sites have been linked to RA pathogenesis [3]. In the study conducted previously when fecal microbiota in patients was analyzed, 16S ribosomal RNA gene sequencing was used to the microbiota in patients with new onset (untreated) RA, chronic (treated) RA & healthy controls [4]. The patients with recent-onset RA had an increased abundance of Prevotella & a decreased abundance of Bacteroides compared with patients with chronic treated RA & healthy patients. According to previous studies *Prevotella copri* as a most abundant Prevotella species in a majority of patients with recent onset RA [3].

New - onset untreated rheumatoid arthritis (NORA) was defined as disease duration of a minimum of 6 weeks and up to 6 months since diagnosis, and absence of any treatment with Disease modifying anti-rheumatic drugs (DMARDs), biologic therapy or steroids. Chronic RA was defined as any patient meeting the criteria for RA whose disease duration was a minimum of 6 months since diagnosis. Prevotella is Gram negative, obligately anaerobic, nonspore forming, non-motile pleomorphic bacilli. Pigmented prevotella species: Prevotella melaninogenica, prevotella pallens, Prevotella loescheii, Prevotella denticola, Prevotella tannerae. Non pigmented prevotella species: *Prevotella copri*, Prevotella bivia, Prevotella oralis, Prevotella oris, Prevotella veroralis, Prevotella zoogleoformans, prevotella buccae.

Prevotella copri is an intestinal bacterium & its anaerobic qualities allow it to grow successfully in the human gut. *Prevotella copri* is present in the intestinal microbiome of: - 75% of those with new onset, untreated RA - 12% of those with chronic treated RA- 38% of people with Psoriatic arthritis - 21% of healthy controls. This study has been taken up to explore the role of *Prevotella copri* as an intestinal coloniser in NORA patients attending JSS Hospital.

2. Materials and Methods
2.1 Materials
Out-patient and In-patient subjects with clinical diagnosis of new onset RA attending JSS Hospital during the study period

2.2 Sample Size
30 cases of new onset Rheumatoid arthritis and 25 Healthy controls.

2.3 Method of Collection of Data
Type of study: Comparative Cross-sectional Study.
Study period: March 2016-March 2017

2.4 Inclusion criteria:
New onset RA with 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.

2.5 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 Probiotics
- Any gastrointestinal tract surgery leaving permanent residue (gastrectomy, bariatric surgery, colectomy), or significant liver, renal or mouth container.
  1. Sample collection: Feces samples is collected was a clean wise mouth container
  2. Transportation: Approximately 250-300 mgs sample was transferred into 2 vial containing 1ml RTF each with the help of wooden / plastic spatula

2.7 Culture Media's Used

- Reduced transport fluid.
- Kanamycin and Vancomycin blood agar

2.8 Culture

- Immediately at laboratory serial dilutions of sample in RTF was done i.e., 1:10, 1:25, 1:100
- If not possible to process the sample immediately, sample can be stored at -4ºC for one day
- Another aliquot of sample was stored at -80ºC for molecular analysis
- The serially diluted sample was inoculated on to Kanamycin and Vancomycin Blood Agar.
- The respective plates were incubated anaerobically by gas pak method for 72 hours.
- At the end of incubation, Gram smear and motility test were done from all non-pigmented, translucent colonies and whenever non-motile short GNB were seen, they were subjected to biochemical tests as per table 1 to detect Prevotella species and *P. copri*.

3. Results

The present study entitled **"**Study of association of intestinal Prevotella in New onset Rheumatoid Arthritis (NORA)" was carried out in Department of Microbiology, J.S.S. Hospital from March 2016 to March 2017. 30 stool samples for NORA cases and 25 for healthy controls formed the material for the study.

3.1 Age Distribution of Patients

In the study population 5(17%) patients were in the age group 20-30, 10(33%) between 31- 40, 8(27%) in the age group 41-50 and 7(23%) between 51 – 65. Maximum number of patients were from the age group of 31- 40.

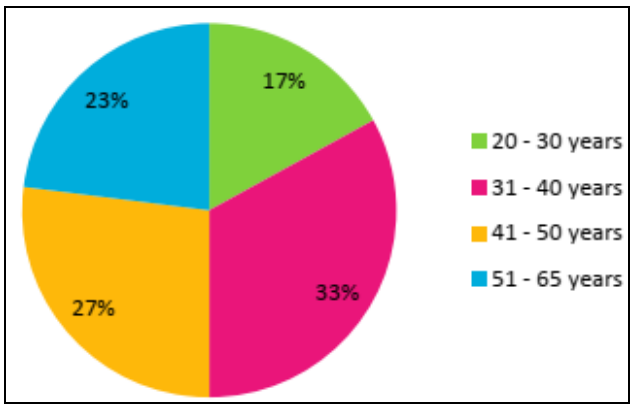

Fig 1: Age distribution of patient

3.2 Sex Distribution of Patients

Out of 30 patients 25(83%) were females and 5(17) were males.

3.3 Stool Culture

Anaerobic cultures of stool samples were negative for *P. copri* both in NORA and healthy controls.

3.4 RF test & Anti CCP ELISA for NORA cases

RF test and Anti CCP ELISA were also done for all cases of NORA. 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.

Table 1: Test wise distribution

	Positive	Negative
Rheumatoid Factor	17	13
Anti CCP ELISA	7	23

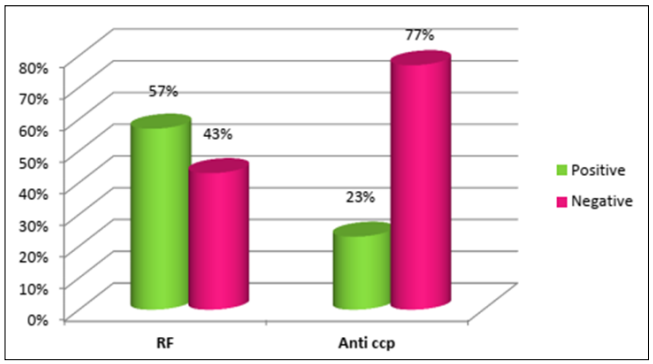

Fig 2: Test Wise Distribution

4. Discussion

Rheumatoid arthritis (RA) is a prevalent systemic autoimmune disease, caused by a combination of genetic and environmental factors. If left untreated, can lead to chronic joint deformity, disability and increased mortality. Despite recent advances towards understanding its pathogenesis, the aetiology of RA remains elusive. Many genetic susceptibility risk alleles have been discovered and validated [5] but are insufficient to explain disease incidence. RA is therefore a complex multifactorial disease requiring both environmental and genetic factors for onset and progress [6].

Among environmental factors, the intestinal microbiota has emerged as a possible candidate responsible for responsible for the priming of aberrant systemic immunity in RA [7]. A marked association with anaerobic bacteria, especially the Prevotella species predominantly *P.copri* has been observed in many studies [8].

With this background, the current study was taken up to detect similar association of gut microbiota in the newly detected cases of RA at JSS Hospital Mysuru, during the study period of 1 year.

There were 30 cases of new onset RA in the study 25 being from female subjects. RA is known to be a disease of the female sex. 19% of the patients had vegetarian food habits while the rest were of mixed diet. Similar results were observed in the study by Carlotta De Filipp *et al.* [9]. They found more association of *P. copri* in RA patients who were vegetarian.

Prevotella copri could not be cultured from any of the samples. This may be attributed to *P. copri* in small numbers in the feces samples or overgrowth of commensal flora. The technique was refined and selective media was included, inspite of which the culture was negative. The standard strain of *P. copri* could not be procured due to the exorbitant cost by reference laboratory. *P. copri* was detected by PCR in 19 cases out of the total study population of 30 cases of NORA (63%). Only 7 out of the 25 healthy controls tested positive for *P. copri* DNA by PCR. In a study conducted by Jose U Scher *et al.*, [8] 75% of NORA patients and 21.4% of healthy controls carried *P. copri* in the intestinal microbiota.

Rheumatoid Factor and Anti CCP testing was done by ELISA for all the NORA cases, out of which 17 showed positive to RF and only 7 showed positive result to Anti CCP. Anti CCP has been quoted as a marker of active rheumatic activity in literature [10]. It is a possibility that with progress of the disease the antibodies may be detected in more number of samples. In a study conducted by Francesca Ingegnoli *et al.*, RF sensitivity was upto 60% - 90%.

In the present study out of 25 healthy controls 7(28%) patients had *P. copri* in the gut microbiota. In a study conducted by Donghyun Kim *et al.*, [11] P. cori was present in healthy controls and in another study done by Jose U Scher *et al.*, [8] 21.4% of healthy controls carried *P. copri* in the intestinal microbiota.

5. Conclusion

There was a significant association of *Prevotella copri* with new onset Rheumatoid Arthritis as compared to healthy adults in the current study. The organism's role may be triggering the immuno-pathogenesis of RA, the aetiology of which is due to environmental and genetic factors combined together. 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. Anti CCP test was positive in only 23% of the study population. This could be due to the fact that new onset cases were included and antibodies may be detected at later stages of disease. However RF test was positive in a significantly higher number of cases (57%).

Prevotella copri nucleic acid could be detected in a significant number of RA samples by PCR as compared to healthy controls (p value = <0.0001). 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 aetio pathogenesis of Rheumatoid arthritis.

Acknowledgments

The author profusely thankful to the Department of Microbiology, J S S Medical College, Mysuru, Karnataka for providing facilities for this research work at the Department

Conflict of interest statement

We declare that no conflict of interest.

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