SUMMARY AND
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This thesis aimed to design and develop enhanced immunoassays for the early diagnosis of UC. Furthermore, the aim was to develop and validate an algorithm for the efficient diagnosis of patients with UC. The first objective focussed on studying the autoimmune profile of patients with UC. In spite of years of research focussed into the epidemiology of UC, its exact causes yet remain to be conclusively elucidated. Epidemiological studies based upon the geographical, ethnic, hereditary, genetic as well as lifestyle related factors are now shedding light upon the causative agents, risk factors, diagnosis, prognosis and treatment of Ulcerative Colitis. The important role played by environmental factors related to geographic location, time of occurrence, age of onset and food habits is being highlighted by recent researches in the field of incidence and prevalence of UC. This thesis has highlighted the role of many of the epidemiological factors in the expression and progression of UC. The continuously most predominant factors that impact the nature of UC in patients, as have also been demonstrated in the present study, are smoking, eating habits and physical exercise.

The second and third objectives emphasised on the development and validation of Immunofluorescence based serological assays for the efficient diagnosis of UC along with comparison of their performance characteristics to determine the assay best suited for the Indian setting. Three indirect immunofluorescence based assays were developed and validated for the diagnosis of UC. The assays showed enhanced sensitivity and specificity at fraction of the cost of imported ANCA diagnostic assays. The novel UC ANCA assay developed and validated presented the best performance characteristics amongst all the in-house developed assays. In a country of more than 1.2 billion population, this assay will open up new avenues in autoimmune UC diagnostics to understand its pathophysiology and etiology. Further research in this topic may be carried out in elucidating the specific antigen or a panel of antigens corresponding to atypical p-ANCA which will dramatically change the way we look at UC and usher in a new era in its diagnosis and treatment.

Above investigation represents the outcome of various experiments based on the strategies employed to achieve the objectives set for present dissertation. Salient findings are given below:
1) 45 UC cases diagnosed at MGM Hospital, Navi Mumbai along with 45 age and gender matched healthy individuals were included in the prospective case-control study.

2) A survey of the subjects comprised of questions pertaining to their demographic details, health, disease record, smoking, food habits and major stressful events was undertaken. These questions were designed to establish a possible correlation between the disease occurrence or prognosis and the mentioned risk factors.

3) The first occurrence of UC was seen in the age group of 30 to 45 years, which corroborates the findings of earlier studies.

4) Tobacco in the form of smoking was found to reduce the severity of the physical symptoms of UC. Patients consuming tobacco reported that their symptoms were better manageable.

5) Physical exercise was seen to play a positive role in management of UC symptoms amongst patients. The patients undertaking regular physical exercise noted that had more manageable symptoms.

6) Sugar and fast food were seen to be involved in aggravating the symptoms of UC. Majority of the participants used extra sugar in their food as well as consumed fast food more than once a week.

7) In order to establish the fact that UC antigens are localized in the inner periphery of the nucleus, Spectrophotometric analysis, UC-ELISA and Dot Blot analysis of the specimens were performed.

8) Based upon the available literature and clinical validation, an algorithm for UC diagnosis was established and validated in Indian Population by testing the UC specimens with the commercial assays available in the market.

9) In-house ANCA IIF assay was developed and validated against commercially available BioRad ANCA IIF assay.

10) In-house UC ELISA assay was developed and validated against commercially available BioRad ANCA IIF assay.

11) In-house modified UC ANCA IIF assay was developed and validated against commercially available BioRad ANCA IIF assay.
12) Performance characteristics of all the in-house developed assays were compared to those of BioRad ANCA assay to determine whether the assays were better than the commercial assay.

13) The novel modified UC ANCA IIF assay developed and validated presented the best performance characteristics amongst all the in-house developed assays.

14) The novel modified UC ANCA IIF assay was superior as compared to the commercially available assays in serological diagnosis of UC because of its unique quality to differentiate between atypical pANCA related to UC and other ANCA related diseases based upon nuclear fluorescence.

In conclusion, this study established efficient methods for diagnosis of UC in Indian population. A systematic diagnostic algorithm along with three indirect immunofluorescence based assays were developed and validated for the diagnosis of UC. The assays showed enhanced sensitivity and specificity at fraction of the cost of imported ANCA diagnostic assays. The novel modified UC ANCA IIF assay developed and validated presented the best performance characteristics amongst all the in-house developed assays. This study will have significant implications for the optimal cost-effective approach for detecting ANCA antibodies in patients. India being a developing country, with almost half of its population living near the poverty line, majority of the population can rarely afford expensive tests for diagnosis of diseases followed by even more expensive treatment. It is essential to note that the projected cost of the in-house developed modified UC ANCA IIF Assay is one-tenth of the average cost of an ANCA-IIF Assay imported from western countries. Our study therefore will have significant cost implications for diagnostic laboratories, particularly due to the increasing number of patients now being screened for ANCA in India. The cost efficiency of modified UC ANCA IIF assay will prove to be a decisive factor in including this test in the first line of tests for the early diagnosis of UC. In a country of more than 1.2 billion population, this assay will open up new avenues in autoimmune UC diagnostics to understand its pathophysiology and etiology.

The nucleus extraction technique developed as a part of this project can be used in a UV based POC assay wherein the UC diagnosis will be enabled by the bedside within a few simple steps. The challenges faced by this approach are, adaptability of
the nuclei to a POC platform, number of steps required to reach the end result and sensitivity of the UV based instrument to detect fluorescence. This project has established beyond doubt that the UC antigens are located near the inner periphery of the nuclear membrane of the neutrophils. This fact can be further studied by fractionating the nuclei further and separating its proteins using affinity gel filtration and elution techniques. Thus we may be able to narrow down to the fraction of proteins which shows maximum reactivity to the ANCA antibodies. The further analysis of this protein fraction by mass spectrometry may enable us to determine the individual proteins present in the fraction. These individual proteins may be purified and analyzed for their antigenicity against ANCA antibodies. Thus we may be able to get an antigen more specific for UC. The challenges which may be faced by this approach are, loss of antigenicity of the antigen during processing, if antigen is a protein-DNA complex it might not retain its native antigenic conformation, there might be a panel of antigens which are not very specific to UC but are present in variable concentrations in the human cells.

Thus this project has laid down foundation for further research in the field of Ulcerative colitis, which is rapidly becoming a cause of grave concern in India. The diagnostic techniques developed within this project will immensely help in detection of Ulcerative colitis at the primary stages itself with better accuracy and within less span of time. The cost efficiency of these assays will enable them to be put to us for the underprivileged masses for whom expensive diagnostic tests are out of bounds. These assays will enable us to better document cases of UC and help in carrying out further research into the epidemiology and pathophysiology of UC.