1. Introduction

Oral health is a determinant factor for quality of life; an important component in maintaining the physical, mental and social comfort, being necessary to evaluate it permanently and establishing new methods of restoring and maintaining it in normal limits. The public health problems associated with oral disease are a serious burden on countries around the globe. The prevalence of oral diseases is increasing in low and middle income countries. The oral disease burden is significantly higher among poor and disadvantaged population groups.

Yeasts are opportunistic pathogens and common members of the normal oral flora in humans. However, the source of early yeast infection, time and stability of colonization of yeast species are poorly understood. Yeasts have attracted growing interest among researchers due to the increased incidence of severe oral candidiasis. Numerous epidemiological studies showed that diseases such as oral candidiasis, tooth decay, dental caries, plaque and gingivitis are among the most common afflictions of mankind. Among these, candidiasis contributes to the major important oral disease in immune suppressed patients. It’s being achieved by the alteration of normal oral environment that favors the growth of Candida species that transforms from a normal harmless commensal to a pathogenic strain. When the host’s innate and adaptive immunological defense mechanisms are weakened, Candida species becomes an opportunistic fungal pathogen, resulting in yeast overgrowth and penetration of the oral tissues. Such changes are called predisposing factors which makes the oral tissues susceptible to the Candida infection.

Candida albicans is the leading cause of fungal infections in the oral cavity, representing 50 to 70% of isolated yeast. Although C. albicans is the most prevailing species, various epidemiological and clinical studies are available to exhibit the high prevalence of non-albicans species such as C. tropicalis, C. parapsilosis, C. krusei, C. kefyr, C. glabrata, C. guilliermondii, C. dublindiensis and C. lusitaniae which are gradually increasing and persists in the human oral cavity as commensal. The two Candida species viz., C. tropicalis and C. krusei constitute the majority of clinically significant yeasts next to C. albicans isolated in most of the hospitalized clinical cases.

The Organisms existing in the oral cavity are called oral microbiome and the oral fungal microbiome is called mycobiome and considered as a vital element of health and disease. The evidence from various epidemiological and clinical studies are available to exhibit the high prevalence of Candida among the several fungal genera of oral mycobiome.
Several species of *Candida* include *C.albicans*, *C.tropicalis*, *C.krusei*, *C.glabrata*, *C.dubliniensis*, *C.paropsilosis*, *C.lusitaniae*, and *C.guilliermondii* persists in the human oral cavity as a commensal. Among these *C.albicans* is the most virulent and prevalent species, although other species such as *C.krusei* and *C. tropicalis*, are emerging pathogen in these days, most frequently associated with normal oral carriage in humans, occurring in the oral environment of up to 80% of healthy individuals.

The clinical manifestation of oral candidiasis is the presence of white pseudomembranous patches or plaques on the surfaces of the tongue and other mucous membranes of the oral cavity. Affected patients complain of tongue pain, a burning sensation and symptoms of taste disorders; therefore, oral candidiasis has an important effect on the quality of life. Based on the severity of infection they are classified as follows: Acute pseudomembranous candidiasis (thrush) is characterized by leukoplakic plaques that appear as white patches and that can be scraped away to expose an erythematous base; hyperplastic candidiasis, by confluent leukoplakic plaques that cannot be scraped away; angular cheilitis, by leukoplakic and erosive lesions at the lip commissures; and atrophic candidiasis, by painful erythematous mucosal lesions, frequently located beneath dentures.

The disease is rare in healthy adults where as it is up to 5% of newborn infants, who become infected during birth from mothers with vaginal candidiasis, and are susceptible due to their immature immune system and instead tends to develop in older patients with a declining immune function which is an important predisposing factor. Denture wearers are susceptible to denture stomatitis, one of the major types of oral candidiasis and immunological impairment also increases infection rates in patients receiving long term steroid or antibiotic therapy or those with diabetes (endocrine disorder) or blood diseases. HIV infection in which oral candidiasis is a sign of possible clinical succession to AIDS. Immunocompromised patients are more susceptible to oral candidiasis since the host fails to recognize the pathogen due to their compromised immune system and facing problems with activating antifungal defense mechanisms.

Predisposing factors comprise of immunosuppressive therapies, smoking, use of dentures, xerostomia, anaemia, endocrine disorders, and primary and acquired immunodeficiency and a high carbohydrate diet. Immunosuppressive therapies using corticosteroids drugs, radiation therapy and use of broad-spectrum antibiotics has also been an important factor for *Candida* overgrowth which modifies the normal oral microbiota. Saliva is important in helping of controlling yeast growth in the oral cavity. Hypofunction of salivary gland so-called Xerostomia is another important predisposing host factor for the
proliferation of the yeast *Candida*. It reduces the flow of saliva and decreases the pH of the oral cavity.

Interestingly, the growth of *Candida* can also be regulated by antimicrobial proteins such as lactoferrin, sialo peroxidase, lysozyme, anti-*Candida* antibodies and phagocytes present in the saliva. It is a more common symptom in the aged and patients with diabetes mellitus, Sjögren’s syndrome and those undergoing head or neck radiation therapies. The Candidal growth is augmented by glucose present in saliva since it can be consumed as an energy source which improves the adherence capacity of *Candida* cells to oral epithelial cells. Chronic type of candidiasis occurs in the cases of genetic autoimmune diseases such as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy which is concerned with progression of oral cancer. In addition, predisposing factors comprise of Immunosuppressive therapies, smoking and anaemia has also been an important factor for *Candida* overgrowth which modifies the normal oral microbiota.

Various virulence factors are contributing to the colonization and pathogenicity of *Candida* infection, including the expression of adhesions (Als1p-Als7p and Asl9p, Hwp1p, Int1p, Mnt1p) and invasins on the cell surface, yeast-hyphal morphogenetic transformation, the formation of biofilms, phenotypic switching and secretes organic acids during infection which results in a decrease in the pH of the surrounding. These acids either exhibit cytotoxicity to the mucosa or activate the production of the hydrolytic enzymes to cause inflammation of the mucosa. Among the various factors, extracellular hydrolytic enzymes of which SAPs (secreted aspartyl proteinases) are considered to be one of the major virulence factors play a major role in over growth of the *Candida*, since these enzymes pave way to adhere, penetrate and for tissue invasion.

Phospholipase enzymes, another important virulence factor, are associated with the function related to host cell damage, adherence and penetration. There are four secreted phospholipids, A to D (PLA, PLB, PLC and PLD). Furthermore, *Candida* is able to acquire elemental iron from host tissues through haemolysin production, iron chelators (siderophores) and iron-transport proteins, which then is used by the fungus for metabolism, growth and establishment of disseminated candidiasis. These virulence factors contribute to the pathogenicity of the organism in the penetration of the pathogen into the tissues to get nutrients, escape from the host defence and blocks the detection by the host immune system. To establish a successful infection, *Candida* has the ability to adapt to diverse host niches which are supported by a wide range of virulence factors and fitness attributes.
Currently available common method of treating oral thrush is to use a medicated liquid that is swished around the mouth and swallowed, or a lozenge that is sucked, dissolved in the mouth, and swallowed. Patients with refractory candidiasis may require systemic drugs. The antifungal drugs of choice are:

- the polyenes (e.g., nystatin and amphotericin B) causing disruption of cell membrane
- azoles (e.g., miconazole, clotrimazole, ketoconazole, itraconazole, clotrimazole, voriconazole, posaconazole and fluconazole) inhibiting ergosterol synthesis
- echinocandins (e.g., caspofungin, micafungin, anidulafungin) which inhibits beta 1,3-D glucan synthesis

In addition to the above antifungals, allylamines, and the DNA analog 5-flucytosine are also included for the treatment of oral candidiasis.

There are over the counter antimycotic drugs available in the market for the treatment of oral candidiasis. But with long term and improper use they result in antimycotic resistance which is quite a major risk in the treatment. Therefore, research community is very solemn in looking for the alternatives to these antimycotics. To overcome these problems, antibody based therapeutics has come of age implicating the researchers to the development of novel drugs. Oral immunotherapy by passive immunization with specific antibodies is a strategy that has been actively practiced by the research community for the last two decades. Since it is possible to produce antibodies in chicken against a vast array of antigens and epitopes, there exists scope for raising antibodies against any number of bacterial, viral, or biological antigens.

Antibodies presently available for research, diagnostics and therapy are mostly mammalian monoclonal or polyclonal antibodies. Bigger animals such as horses, sheep, pigs and also rabbits and guinea pigs were used for the production of polyclonal antibodies, while mice and rats were used as a source of spleen for the production of monoclonal antibodies. Nowadays, most frequently chosen mammals for polyclonal and monoclonal antibody production are rabbits and mice respectively. Available techniques and concern for animal rights enhance the interest in developing alternative methods for the production of antibodies. The most important aim of animal welfare is to reduce painful manipulations. Several important advantages are offered by antigen-specific antibodies produced in chickens over antibody production in mammals.
Introduction

In view of the fact that cross reactivity of chicken IgY does not happen with mammalian IgG and does not bind bacterial or mammalian Fc receptors, non-specific binding is reduced and the need for cross species immune absorptions also is eliminated. In addition, eggs from hyper immunized chickens serves as a better source for a continual and daily source of polyclonal antibodies throughout the year. IgY technology fulfils this requirement in that chicken antibodies can be easily sampled by a non-invasive method based on the simple act of collecting eggs. IgY technology also offers outstanding economic advantages because maintenance of hens is cheaper than rabbits. Furthermore, an extraordinary amount of antibodies can be produced from only one hen approximately 17-35g of total IgY/Chicken/Year of which 1-10% can be expected to be antigen specific. This huge quantity of available antibodies opens door for new fields of IgY applications, such as immunotherapy and immune prophylaxis for several viral and bacterial infection in veterinary and human medicine.

There are many reports suggesting the possibility of preventing oral candidiasis (active immunization) using antigen specific chicken egg yolk immunoglobulin (IgY). IgY specific to Candida sp., can prevent the oral candidiasis by adhesion to the host cells, thereby preventing candidiasis from further progression. Since adherence to the host cells is an essential condition required for the successful establishment of the virulence properties of mucosal pathogens; thus, intrusive with the adherence of a Candida put off or hold up the colonization. Hence the present study was carried out to generate antibodies against Candida albicans, Candida tropicalis and Candida krusei and to test the efficacy of these antibodies in controlling oral candidiasis in vitro and in mice model.

Objectives

- To generate and characterize Chicken egg yolk antibodies (IgY) against Candida albicans, Candida tropicalis and Candida krusei in white leghorn Chickens.
- Evaluating the potential effects of anti-Candida albicans, anti-Candida tropicalis and anti-Candida krusei specific IgY antibodies against Candida species by in vitro assays.
- To determine the neutralization effects of anti-Candida albicans, anti-Candida tropicalis and anti-Candida krusei IgY to prevent oral candidiasis in mice model by in-vivo study.
Introduction

- To compare the anti-Candidal activity of commercially available mouth rinse against *Candida albicans*, *Candida tropicalis* and *Candida krusei*.
- To formulate an oral composition comprising of the generated anti *candida* sp., chicken egg yolk antibodies and to evaluate its effects *in vitro*. 