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Comparative analysis of oral *Candida* albicans rate in healthy people and people with HBSAg$^+$ referring to blood transfusion organization in Ahvaz in 2008

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Abstract

Background and Aim: The hepatit is one of the most common and serious elements of hepatitis in the world. The cause of the infection is unknown but previous findings indicate that disturbance in function of immune system, B lymphocyte, Foxp3, CD4$^+$ and CD8$^+$ are the probability causes. The candida species are the natural mouth florae in very people and exist in the mouth of the carrier people in lower concentration about 200-500 cells in one milliliter of saliva. The goal of this search was to comparison the level of oral *Candida albicans* in healthy and HBsAg$^+$ persons referred to Ahwaz Transmittal Blood Organization in 2008.
Materials and Methods: This Case-Control study was carried out on 56 persons (28 persons in each group) referred to Ahwaz Transmittal Blood Organization. These individuals were divided into two groups of healthy and HBsAg$^+$. The two groups were without a history of anxiety and alcohol and cigarettes or drug consuming. Samples were taken two weeks after the mention education from their saliva. The saliva samples were cultured in chromagar media and placed in the incubator for 48 hours. Finally the number of colonies counted by colony counter machinery. The data were analyzed with Chi-square test.
Results: In healthy group persons there were 2 carriers (7.1%) and in HBsAg$^+$ group persons there were 11 carriers (39.3%). A significant correlation was observed between HBsAg$^+$ and *Candida albicans* (P-value=0.004).
Conclusion: The number of Candida albicans carries in HBsAg+ persons was more than healthy persons.

Keywords: *Candida albicans*, Hepatitis B, HBsAg$^+$, Saliva

Introduction

Hepatitis B virus is one of the main problem of global public health (1-7) by which more than 2 billion people has been infected in whole world (2,5). In spite of easy access to very effective vaccine for HBV, it is estimated that there are 350 million people that are transporter of this disease globally (2,8). People who are infected by

HBV for a long time, they are more at stake of being suffered from liver disease including *liver* cirrhosis and liver cancer. Death rate estimated more than 780000 years in a year (2). Prevalence rate of HBV infection is different in whole world. Iran as a region, prevalence of HBV is categorized as low-average (2-4/99). Prevalence rate of HBV infection was 1/7% in Iran in 1990s. Based on study in 2016, prevalence rate of HBV in Iran is 3% (5). But regional studies in different provinces reported prevalence rate as 1/7% in last decade (2). Currently there is no assured medication against infection of HBV (9). And nowadays vaccination is most effective and economic tool for HBV prevention (10,11).there is no consistent cause for this infection however data shows disorder in immune system performance and disorder in lymphocytes T ،Foxp3 ،CD_8^+ ،$CD_4^+$are common cause of this infection (9). Specifically, it is indicated that CD_4^+,CD_8^+ responses of lymphocytes T cells has main role in causing disease (7).

On the other hand candida is outstandingly one opportunistic infection factor (12). Cloning caused by candida in mouth of healthy people is 25-30%. And this rate was increased in people who suppressed immune system (13). Different types of candida are natural oral flora in most people and it is existed in mouth of healthy transporters with less density as 200-500 cells in each cc of suliva (12). Host immune response (and also variation of pathogenic factors causing them) has main role in development of candida infection (13).

According to high prevalence rate of HBV transporters, candida role and chronic infection of Hpatitis B and C as *carcinogen (14), elimination of* extraneous infections by lymphocytes T and possibility of disorder in lymphocytes T performance in people with $HBSAg^+$ we decided to study prevalence rate of candida in people with $HBSAg^+$ and role of lymphocytes T in elimination of this infection.

Methodology of the study

This study was Case-Control performed on 56 people referring to blood transfusion stations in Ahvaz who aimed at blood donation in 2008. They were divided into two 28 people group.

First group included 28 person with $HBSAg^+$ while positivity of the result was approved by the blood transfusion organization and infection professional. These people systematically had no problem but $HBSAg^+$ and did not consume any medicine and they had no record of Alcohol and cigarette consumption. They were studied considering depression and anxiety problem because we did not want depression effect on suliva has impact on our study. In addition, people who had less oral health were eliminated from the study. by last year student in dentistry coarse, 1 cc of these people's suliva was taken without washing the mouth and in fasting condition and kept in streel pipes. Sample was cultivated in Chrome agar cultivation environment and after 48 hours in 37 centigrade degree, colony rate of *Candida albicans* was reported by Colony count machine. In addition, color of *Candida albicans* colony in cultivation environment of chrome agar was light green while C*andida tropicalis* was blue and *Candida crose* was pink. Second group or control group (28 people) were whom that had received Hepatitis B vaccine while their anti-body titr was positive and they had no record of systemic disease or anxiety and depression. People with $HBSAg^+$ who were approved by blood transfusion organization were interred to the study by written consent and being confident about their information privacy.

Mixtures of chrome agar cultivation environment of candida

This colorful cultivation environment that we used for our research was new production of France. Each box had 47/7 G weight while 15 g/l was agar, 10/2 g/l was Piton, 22 g/l was colorful mixtures, 0/5 g/l was chloramphenicol. This cultivation environment has 6/1 pH. Color of colonies of *Candida albicans* colony in cultivation environment of chrome agar was light green while c*andida tropicalis* was blue and *Candida crose* was pink.

Preparation of chrome agar cultivation environment of candida

Before sampling of people, chrome agar cultivation environment of candida was tested by isolations. After assurance from cultivation environment, sampling was performed. In order to provide one litter of distilled water from cultivation environment there is need to solve 47/7 g cultivation environment powder with one litter distilled water. First, we weighted cultivation environment powder by digital scale and poured it in Elren with distilled water by spatula and placed it in hot plate. During heating, there is needed to be careful that temperature of cultivation environment is not higher than 100 centigrade degree. Heating cultivation environment must be continued in order to create clear cultivation environment. After that, erlen with cultivation environment is taken from hot plate until the temperature is 45 centigrade degree. Then cultivation environment beside oven flame was distributed inside of sterile plates. Then plates were placed on plane area in cold condition for being solid. After that, plates were placed in refrigerators. Maximum time that this cultivation environment can be kept in the refrigerator is one months. In case of keeping it in room temperature, cultivation environment cannot be preserved more than one day.

Statistical methods of analysis

Chi- square test was used for comparing relation in both case and healthy group.

Results

In this study, in control group (n=28) Ab titr was higher than 10MIU/m. case group (n=28) had no problem but $HBSAg^+$.

Both groups each one included 21 male and 7 female with average age of 44/3 in control group and 45/1 in case group had no significant difference. Numbers of *Candida albicans* in one cc of sample suliva in healthy group (numbers of *Candida albicans* colony is less than 200 col/ml) and transporter (numbers of *Candida albicans* colony is less than 200-500 col/ml) is shown in table 1.

Table 1: numbers of *Candida albicans colony* in both control and case group

Total	*Candida albicans*		groups
	Non- transporter	Transporter	
28	26	2	healthy
28	17	11	$HBSAg^+$
56	43	13	Total

Results obtained by Chi-square indicated that transporter relations in healthy group was 7/1% and in group with $HBSAg^+$ was 39/3%. Transporter relation in healthy group with $HBSAg^+$ group had significant difference (P-value=0/004).

Discussion

As we stated in result part, percentage of transporter people was 7/1% in healthy people and 39/3% in $HBSAg^+$ group. Similar research had been performed which we explain them.

In study by Fider et al, 45 healthy patients and 45 patients with HIV^+ were studied. In this study 2 healthy person had oral candidiasis while in HIV^+ group 17 patients had oral candidiasis which indicated that exposure of oral candidiasis in people with HIV^+ is more than healthy people (16).

In other study by petry et al, saliva sample of 64 patients with HIV^+ were taken. Colonization of *Candida albicans* was observed in 53 person (82/8%). Out of this number, in their saliva sample, 83% had *Candida albicans*, 22/6% had C*andida glabrata* and 11/3% had *Candida dubliniensis* (17).

It is proved that Hepatitis B virus causes disorder in performance of CD_8^+andCD_4^+ cells (18). Disorder in performance of these lymphocytes T cells cause reduction in production of interleukin-2 and INFry so these cells are not activated because two kinds of cells are phagocytosis and main reason of fungal cells colonization. Therefore, by inactivation of macrophage and NKs, colonization of fungal cells is increased. We discuss it in detail.

Assistant CD_4^+ cells cause construction and secretion of different kinds of cytokines when they are exposed with alien anti-gene. CD_4^+ cells are divided in two sub groups based on different *cytokines (18).*

a) One CD_4^+sub group which is called Th1 makes interleukin-2, and interferon gamma. These cells cause activation of CD_8^+ cells and NK cells and macrophages. In virus infections most CD_4^+cells are activated.

b) Second sub group which is called Th2 produces interleukin-4 and 5 which cause activation of lymphocyte B and production of anti body with change of anti-body group (!8).

c) CD_4^+ cells of cytotoxic: these lymphocytes detect cells that are infected by virus with MHC class II and destroy them (19).

CD_8^+*cells*: these cells which are limited into class I MHC are concentrated in place of virus reproduction and destroy cells infected with virus. As activator of macrophage cells and NKs are interleukin-2, and interferon gamma so these cells are not activated. We explain macrophage and NK performances.

Natural killer cells and macrophages: these cells are observed two days after virus infection. These cells have cytotoxic characteristic for cells infected with virus. NK cells are main mediators of cellular Cytotoxic ependant to anti body (ADCC) (19). Advantages of NK cells rather than lymphocytes B and T is that cell without lag phase is for start of performance thus at first of virus inpu into the body, activities are started and limited virus development (20).

Conclusion

Most important results of this research include prevalence of *Candida albicans* in people with $HBSAg^+$ which is more than people vaccinated against Hepatit B. thus in condition of this disease these people are prone to candidiasis.

The comparative study of the level of oral *Candida albicans* in healthy and $HBsAg^+$ persons referred to Ahwaz Transmittal Blood Organization in 2008.

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