Abstract
The skin is the body’s largest organ that hosts heterogeneous inhabitants. Until now, the diversity of the cutaneous microbiome was mainly investigated for bacteria and there is a little information about the skin fungal flora. Also among skin fungal flora, Candida is found as a main member which its distribution is affected by sex, age, climate. In this study, differences in Candida community structure associated with 9 different skin sites of 238 healthy people during 10 months, are described. These subjects were divided by age into 4 groups: infants, children, adults and geriatrics. The collected samples were examined by culture on Sabouraud Chloramphenicol Agar and CHROM-agar Candida. For precise identification of species DNA sequencing was performed where needed. The frequency of Candida species was significantly different between age groups. The most Candida isolations were related to the elderly age group and the fewest in the infants. C. parapsilosis virtually, was the predominant isolated species in all age groups. This study showed no statistically significant effect of the subject’s sex on Candida population resident on human skin surface.

Keywords: skin residents, cutaneous Candida composition, different age groups, DNA-sequencing, culture, microbial epidemiology, Iran

Introduction
Skin as the largest organ of human body is significantly colonized by a variety of bacterial and fungal population.1,2 Today the percent of opportunistic fungal infections is increasing, so when considering the role of skin as a reservoir from which infection of a susceptible host can occur, determining the fungal microbiome specially Candida is important.3 Two of the most factors influencing the complex of inhabitants on the skin are gender4 and age.5,6 There are more data concerning bacterial cutaneous flora than fungal flora, and among fungal flora, Candida species may be one of the important opportunistic agents residing on the skin with the potential to create different kinds of candidiasis.7,8

There is little information about candida distribution inhabiting on human skin in the word, and our information is related to more than half a century ago. It should be noted, this is the first study about this subject in Iran.

The purpose of this study were to create new information about the type and percentage of candida population in each site of the human skin, attempting to determine what influences the individual’s age and sex have on the composition and percentage of cutaneous candida community in Iranian people. As we know, Iran is a tropical country and fungal organisms can significantly growth over this condition.

Materials and methods

Ethics statement
This study was approved by ethical committee of Tehran University of medical science. A written informed consent was obtained from all subjects or their guardians prior to sample collection. All data were de-identified.

Sampling
A total of 238 healthy people, in four age groups, were studied. This subjects were including 119 males and 119 females, with equal gender distribution in all age groups. All samples were selected from 5 areas of Tehran (North, South, East, West and Center of these city). The distribution of subjects in each group was as follows:

I. 55 healthy, full term babies between the ages of 4 to 15 days, including infants referring to the health house.

II. 60 children aged between 1 and 12. Most were drawn from schools and kindergartens.

III. 62 healthy adults, aged between 18 and 45 years. This group was composed of students of Tehran University, factory workers, health workers and house wives.

IV. 61 old people over 60 years age which often consist of retirees.

At the time of sampling, the age and gender of each subject were recorded. The subjects haven’t washed their hands, feet, or other areas just before sampling and the sampling was made after the activities of the day. The following areas were sampled by means of a cotton-tipped swab moistened with sterile saline physiology: the forehead, dorsum of hands, dorsum of feet, finger nails, toenails, the axilla, the groin, the interdigital spaces of hand and foot and the submammary space in women.
All swabs were cultured on SC (Sabouraud Chloramphenicol Agar, Merck, Germany). All cultures were incubated in 25°C for 4 days. Isolated colonies were identified by growth on Corn Meal Agar Tween 80 (Micro media, Hungary) and CHROMagar Candida (Paris, France) in 30°C after 4 days. Chlamydospor formation by C. albicans on cornmeal agar can be a differential factor for this species. In this study for a correct determination of samples which were not detectable by mycological techniques, DNA sequencing was performed.

Molecular technique

DNA extraction: An aliquot of 100μL of cell suspension was transferred to microtubes and incubated at 100°C in a boiling water-bath for 10min, then centrifuged at 5,000g for 5 minutes. The upper aqueous layer (containing the DNA) was carefully transferred to a clean tube and was used for PCR.

PCR conditions and sequencing: PCR amplification of ITS1-5.8S-ITS2 rDNA regions was achieved using the ITS1 (forward, 5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (reverse, 5'-TCC TAT TGA TAT GC-3') primer pairs (MWG - Biotech AG, Germany). To amplify ITS domains, PCR amplification was performed in a final volume of 50μL. Each restriction consists of 2μL templates DNA, 0.5μL of each primer at 25μM, 1.25μL of dNTP (BIORON GmbH, Germany) at 5mM, 0.5 Taq DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany) and 5μL 10× PCR buffer. The amplification parameters consist of 35 cycles of denaturation at 94°C for 1min, primer annealing at 56°C for 1min and extension at 72°C for 1 min. In the first cycle, the denaturation step was 94°C for 5min and in the final cycle the final extension step was 72°C for 7min. Amplified products were visualized by 1% agarose gel electrophoresis in TBE buffer (20mmol/L EDTA, 10mmol Tris boric pH 8). Gel was stained with ethidium bromide (0.5μg/ml) and photographed by 300-nm ultraviolet trans illuminator (Cybertech, Berlin, Germany). Positive PCR products were sent for sequencing at Bioneer Advanced Nucleic Acids core facility. The ITS sequences were then parsed from the contig and separately used to perform individual nucleotide–nucleotide searches using the BLASTn algorithm at the NCBI website (“http://www.ncbi.nlm.nih.gov/BLAST/”). Fungal identifications were made based on maximum identities ≥99%, query coverage ≥98% and evaluates 0.0 for culture collection isolates as described previously.

Results

In this study, on each medium that the growth of candida was positive, one isolate was identified. However, from a few mediums related to some sites of the skin, two isolates were detected. Also for determination of 40 samples which were not detectable by culture, DNA sequencing was performed.

Among the studied population, divided into 4 groups by age including infants, children, adults and the elderly, 162 persons were positive for Candida isolation. The highest prevalence of Candida isolation was related to the elderly age group (n=83, 51.2%) and the lowest prevalence was related to the infants (n=11, 6.7%) and showed the age of subjects was significantly effective on cutaneous Candida isolation in regard to anatomic sites, from 300 positive sites for Candida isolation, the highest prevalence of Candida isolation was related to the fingernails (n=53, 17.6%) and the lowest prevalence was related to the sub mammary space in women (n=18, 6%) (Figure 3).

In the infants among 55 cases, the highest prevalence of Candida isolation was related to fingernails with 5 positive cases (9%) and the lowest prevalence was related to interdigital spaces of feet with 1 positive case (4%). Also in this age group from 25 isolated species, C. parapsilosis (n=61, 37.6%) showed the highest prevalence of Candida isolation and C. tropicalis (n=5, 3%) showed the lowest prevalence in both genders (Figure 2).

Considering Candida isolation in regard to anatomic sites, from 300 positive sites for Candida isolation, the highest prevalence of Candida isolation was related to the fingernails (n=53, 17.6%) and the lowest prevalence was related to the sub mammary space in women (n=18, 6%) (Figure 3).

In children group from 60 cases, the highest prevalence of Candida isolation was related to dorsum of hands with 8 positive cases (13.33%) and toenails with 8 positive cases (13.33%) and the lowest prevalence of isolation was related to interdigital spaces of feet with 2 positive cases (3.33%). Also in this age group from 39 isolated Candida species, C. kruzi with 15 isolates (38.4%) was predominant among all species isolated from the anatomic sites of each person.

In adults from 62 cases the highest prevalence of Candida isolation was related to the groin with 10 positive cases (16.1%) and the lowest Candida isolation was related to the interdigital spaces of feet with 5 positive isolates (8%). Also in this age group from 66 isolated Candida species, C. parapsilosis with 23 positive cases (35.3%) was predominant.

In the elderly from 61 cases, the highest prevalence of Candida isolation was related to fingernails with 34 positive cases (55.3%) and the lowest prevalence of isolation was related to the sub mammary space with 14 positive cases (22.9%). Also in this age group from 195 isolated Candida species, C. parapsilosis with 75 positive isolates (38.4%) was predominant among all species.

About Candida isolation from different anatomical sites of the skin in regard to the participant’s gender, from 159 isolated Candida species in males group, the highest prevalence of Candida isolation was related to finger nails with 28 isolates (17.6%) and the lowest prevalence of isolation was related to the interdigital spaces of feet with 11 isolates (6.9%). Also from 151 isolated Candida species in females group, the highest prevalence of Candida isolation was related to toenails with 20 isolates (13.2%) and the lowest was related to the axilla with 12 isolates (7.9%).

Discussion

In this study, we characterized the cutaneous Candida composition and the percentage of candida species inhabiting on each site of human skin by focus on show the effect of the individual’s age and sex on it. Our study showed that the lowest prevalence of candida isolation is related to the infant age group, this finding may be a result of this fact that the cases were at the age of 4-15days and the chance of skin colonization with candida during this time is less than other age groups. Fetal skin is sterile, but candida colonization occurs immediately after birth during vaginal delivery and then colonization occurs through the hands of health care workers, parents and the infant’s contact with objects and equipment related to neonatal care.10 The highest prevalence of candida isolation recorded from the skin of elderly individuals. The main reason for this is possibly due to the development of dry skin and decrease in shedding rate in keeping with the general weakness of the body and probable disorders in immune system of skin in this age group.11 In this work, the cutaneous candida community was similar between males and females and there was no significant difference in candida isolation between the genders. whereas in other surveys about the normal flora of the skin the frequency of candida isolation was significantly greater in females compared to males.7 Changes in sociodemographic and economic patterns, life style and diet over the time could be the reason for the observed differences in this study.6,12 During this investigation the maximum amount of candida isolation in regard to anatomic site of the skin was related to fingernails. Considering further chance for hands in contact with pollution sources and the effect of some epidemiological elements such as occupational factors, the increased chance of yeasts isolation from this anatomic site is explainable. Isolation of C. parapsilosis as the commonest species (35%) in all sites of the skin in our study was noteworthy which was consist with other studies about candida colonization.13,14 As other surveys that determined C. albicans seldom lives saprothetically on the skin1 we found that C. albicans is one of the least inhabitants of the skin, whereas new studied showed among Candida species, C. albicans is still the most common infectious agent isolated from clinical samples in Iran and the word.15,16

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Conflict of interest

The author declares no conflict of interest.

References