Effect of statin therapy on oral *Candida* carriage in hyperlipidemia patients: A pioneer study

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None declared

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Abstract

Background. Hyperlipidemia affects about 25% of the adult population globally. Statins are the most commonly used drugs in the management of hyperlipidemia. Laboratory and retrospective clinical studies have observed the inhibitory effects of statins on the growth of different *Candida* species. The effect of statin therapy on oral *Candida* carriage has not yet been investigated.

Objectives. This pioneer controlled study aimed to assess and compare asymptomatic oral *Candida* carriage in a group of 50 hyperlipidemic patients who were on regular statin therapy (HLS group) and in a control group of 50 subjects, matched in terms of gender, age and dental status, who were hyperlipidemic, but not on statin therapy (HLNS group).

Material and methods. The patients were recruited from the outpatient clinics of 2 university hospitals. The concentrated oral rinse technique was used to isolate oral *Candida* species in both groups. *Candida* species were identified using the germ tube test and the VITEK® 2 system.

Results. The *Candida* prevalence and colony count were significantly lower in the HLS group as compared to the HLNS group ($n = 20$, 40% vs. $n = 30$, 60%, respectively; $p = 0.040$). There was no significant difference in the oral *Candida* prevalence or colony count between different age groups in either the statin or control subjects.

Conclusions. Statin therapy is associated with a reduction in oral *Candida* carriage in both prevalence and the colony count in hyperlipidemic patients.

Keywords: *Candida albicans*, hyperlipidemia, statin therapy, oral *Candida*

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Introduction

Elevated blood cholesterol (hypercholesterolemia) is a common metabolic disorder that affects about 25% of adults globally and 44.3% of adult Jordanians.[1] Statins, such as simvastatin and atorvastatin, are the most commonly prescribed medications to control hypercholesterolemia.[2] Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that interfere with the biosynthesis of cholesterol. The main antifungal action of polyenes (nystatin and amphotericin B) has been shown to be the destruction of ergosterol, a component of the fungal cell wall (the fungal counterpart of mammalian cell cholesterol), leading to the leakage of the fungal cell cytoplasm and cell death.[2]

Candida species are widely spreading opportunistic pathogens that constitute part of the oral ecology in up to 60% of normal individuals, with racial and geographic variations. They can cause superficial or, more seriously, hematogenous infections if the host immune barriers are breached, either on the local or systemic level.[3]

Reports have described several pleiotropic effects of statins apart from their serum cholesterol-lowering action, including anti-oxidative, anti-inflammatory, immunomodulatory, and antimicrobial properties.[4] A review of the literature revealed several studies describing the antifungal effects of statins.[5] Relevant to this, simvastatin inhibited the formation of the *Candida albicans* (*C. albicans*) biofilm in an in vitro study.[6] Several studies have shown the growth inhibitory effects of statins against different *Candida* species.[6–10] On the clinical side, a retrospective multicenter cohort study of 326 patients with candidemia reported candidemia in 13.5% of statin users as compared to 86.5% of statin non-users.[11] Another study included 1,019 diabetic patients who underwent lower gastrointestinal tract surgery; the patients who were on statin therapy had significantly fewer positive cultures for *Candida* species from the samples of blood, urine, sputum, and peritoneal fluid during hospitalization.[12] However, one animal study found that atorvastatin lowered the survival rate in a group of mice infected with *C. albicans* via peritoneal injection as compared to infected mice which were not treated with atorvastatin.[13] Welch et al. reviewed 124 candidemia episodes and concluded that statin use did not alter mortality, the length of hospital stay or intensive care requirement.[14]

To the authors' knowledge, no prospective clinical study has investigated the effect of statin therapy on the asymptomatic oral carriage of *Candida* species. The aims of this pioneer study were to assess and compare oral *Candida* species carriage, i.e., the positive oral isolation of *Candida* species without any clinical signs or symptoms of candidal infection, in a group of hypercholesterolemia patients who were on statin therapy and in a control group of hypercholesterolemia patients, matched in terms of gender, age and dental status, who were not on statin therapy.

Material and methods

Study subjects

The subjects of this study were recruited from the Internal Medicine Clinic at King Abdullah University Hospital (KAUH), the Family Medicine Clinic at Jordan University of Science and Technology Health Center and the Internal Medicine Outpatient Clinic at Princess Basmah Teaching Hospital in Irbid, Jordan, between February 2019 and July 2019. The subjects willingly agreed to participate in the study and signed a formal consent form. The study group was composed of 50 hyperlipidemic patients who were on regular statin therapy to control their hypercholesterolemia, in addition to dietary regime and physical activity, for at least 8 months; they were called the hyperlipidemia statin group (HLS group). The control group consisted of 50 gender-, age- and dental status-matched hyperlipidemic patients whose hyperlipidemia was managed only by diet control and physical exercise. They were not on statin therapy and were called the hyperlipidemia non-statin group (HLNS group). The minimal sample size for this pioneer study was determined after consultation with a biostatistician. Hyperlipidemic patients were identified based on previous serum cholesterol level investigations that were requested and confirmed by their attending physicians.

The inclusion criteria were as follows:
– adult patients diagnosed with hypercholesterolemia;
– non-smokers; and
– dentate subjects with at least 10 teeth.

The exclusion criteria were as follows:
– subjects with a removable dental prosthesis;
– tobacco smokers;
– diabetic or hypertensive subjects;
– subjects with complaints of xerostomia;
– subjects with a history of oral candidosis for the last 6 months;
– subjects who were on antibacterial, antifungal or immunosuppressant therapy;
– subjects who were using an antiseptic mouthwash regularly for the last 6 months; and
– subjects who exhibited mucosal changes suggestive of candidosis, clinically evident calculus or overt gingival inflammation.

The subjects in the HLS group were recruited into the study first. Then, the HLNS subjects who matched the corresponding subjects in the HLS group were included.

Microbiological investigations

The concentrated oral rinse technique described by Samaranayake et al. was used for quantitative and qualitative oral *Candida* isolation.[15] Briefly, each subject was supplied with 10 mL of 0.9% sterile normal saline

in a universal container. They were asked to rinse their mouth thoroughly with the saline solution for one full minute, and then return the rinse into the container. The samples were transported immediately in an icebox to the microbiology laboratory at KAUH for *Candida* species identification. The mouth rinse was centrifuged at 1,700 g for 15 min and the supernatant was discarded. The deposit was reconstituted in 1 mL of 0.9% sterile normal saline and agitated with a vortex mixer (Assistent®; Glaswarenfabrik Karl Hecht, Sondheim vor der Rhön, Germany) for 1 min. Subsequently, 0.1 mL of the reconstituted suspension was inoculated onto a culture plate of Sabouraud dextrose agar (Oxoid, Basingstoke, England) and incubated aerobically at 37°C for 48 h. *Candida* colonies were identified based on the colony color, texture and morphology, and the purity of the culture was determined microscopically, using the wet mount technique with a ×100 objective. The colonies on the plate were counted, and the number was multiplied by 10 to calculate the number of colony-forming units per 1 mL of the rinse (CFU/mL). *Candida albicans* and other species were identified using the germ tube test[16] and the VITEK® 2 system with new colorimetric yeast cards (bioMérieux, Durham, USA).[17]

The study protocol was reviewed and approved by the Institutional Review Board at KAUH (ref No. 1/116/2018) in compliance with the Declaration of Helsinki.

Statistical analysis

The data was analyzed using the SPSS Statistics for Windows software, v. 17.0 (SPSS Inc., Chicago, USA). The Shapiro–Wilk test was used to check for the normality of continuous data. The χ^2 test was used for categorical data. The Mann–Whitney *U* test was used for non-normally distributed data. The level of significance was established at $p \leq 0.05$.

Results

Biographic and clinical data

This study included a total of 100 subjects. Each of the study groups was composed of 27 (54%) males and 23 (46%) females. The mean age in the HLS and HLNS groups was 55.6 ±10.5 years and 55.1 ±10.8 years, respectively, and the age range was 37–80 years.

At the time of the study, 28 (56%) subjects in the HLS group were on simvastatin, 14 (28%) were on atorvastatin, 5 (10%) were on fluvastatin, and 3 (6%) were on lovastatin. At the time of inclusion in the study, 42 (84%) of the HLS subjects were on a 20 mg daily dose, while only 3 (6%) were on 10 mg, 3 (6%) were on 40 mg, 1 (2%) was on 60 mg, and 1 (2%) was on 80 mg.

Oral *Candida* carriage

As presented in Table 1, the prevalence and mean count of the isolated oral *Candida* species were significantly lower in the HLS group as compared to the HLNS group. *Candida albicans* was the most commonly isolated species in both groups. In addition, a lower number of species was isolated in the HLS group ($n = 4$) as compared to the HLNS group ($n = 6$). The prevalence of *C. albicans* was lower, although not statistically significantly, among the HLS group (18%) as compared to the HLNS group (30%).

The statistical analysis showed no significant association between either the prevalence or the mean count of the isolated oral *Candida* species and the subject's age or the duration of statin therapy (Table 2).

Discussion

To the author's knowledge, this pioneer clinical study is the first to investigate the effect of statin therapy on the oral carriage of *Candida* species. The finding that the oral cavity of 60% of the hyperlipidemic patients who were not on statin therapy was colonized by *Candida* is comparable to the 57.7% prevalence previously reported among the general population from the same geographic area,[18] which gives validity to the current results. It may be suggested that hyperlipidemia per se is not a factor that affects oral *Candida* carriage. *Candida albicans* has previously been shown to be the most common *Candida* species isolated from the oral cavity,[3] which is consistent with the results of the present study. The concentrated oral rinse technique used in this study is known for its superiority, both qualitatively and quantitatively, in the overall candidal sampling of the oral cavity.[15] In an attempt to neutralize the possible effects of any confounders, the study group and control group subjects were closely matched for gender, age and the dental status, and strict inclusion and exclusion criteria were implemented.

Table 1. Prevalence and mean count of different *Candida* species isolated from the study subjects

Variable	HLS ($n = 50$)	HLNS ($n = 50$)	*p*-value	Total ($N = 100$)
C. albicans	9 (18)	15 (30)	0.160	24 (24)
C. kefyr	1 (2)	3 (6)	0.617	4 (4)
C. dubliniensis	7 (14)	3 (6)	0.318	10 (10)
C. spherica	0 (0)	5 (10)	0.056	5 (5)
C. krusei	0 (0)	1 (2)	1.000	1 (1)
C. glabrata	3 (6)	3 (6)	1.000	6 (6)
Total	20 (40)	30 (60)	0.040*	50 (50)
Count [CFU/mL]	49.1 ±18.0	162.6 ±28.0	0.0001*	105.8 ±24.0

Data presented as number (percentage) (*n* (%)) or as mean ± standard deviation (*M* ±*SD*). HLS – hyperlipidemia statin group; HLNS – hyperlipidemia non-statin group; CFU – colony-forming unit; * statistically significant.

Table 2. Relationship between the prevalence and mean count of the isolated oral *Candida* species and the patient's age and the duration of statin therapy in the HLS group

Variable		Prevalence	*p*-value	Count [CFU/mL]	*p*-value
Age [years]	<50 (*n* = 18)	8 (44.4)	1.000	36.9 ±11.0	0.950
	51–60 (*n* = 16)	6 (37.5)		26.9 ±5.0	
	>60 (*n* = 16)	6 (37.5)		83.7 ±18.0	
	total (*n* = 50)	20 (40.0)		49.1 ±18.0	
Duration of statin therapy [months]	<24 (*n* = 20)	9 (45.0)	1.000	18.2 ±6.0	0.860
	25–70 (*n* = 19)	7 (36.8)		86.4 ±17.0	
	>70 (*n* = 11)	4 (36.4)		27.3 ±12.0	

Data presented as *n* (%) or as *M* ±*SD*.

The antifungal potential of statins has been described in several animal and laboratory studies.[6–10,19] To date, this study is the first clinical investigation to demonstrate that statin therapy is associated with a significant reduction in oral *Candida* colonization, both qualitatively and quantitatively. The antifungal mechanisms of statins are not clear. Statins lower cholesterol levels in blood through their action on HMG-CoA reductase, an essential enzyme for the biosynthesis of cholesterol. It is widely believed that fungal HMG-CoA reductases may also be inhibited by statins, resulting in reduced levels of cell wall ergosterol (the fungal equivalent of cholesterol), and the subsequent disruption of the synthesis of the candidal cell wall, which leads to the loss of intracellular components and cell death.[5] This hypothesis was indirectly supported by the research which determined that the addition of exogenous ergosterol to the statin–*Candida* assay overcame the inhibitory effect of simvastatin.[19,20] Ting et al. in their systematic review described other possible antifungal mechanisms of statins.[21] Statins may indirectly affect fungal cell signaling, proliferation and differentiation through the inhibition of the synthesis of isoprenoid. In addition, statins can adversely affect the synthesis of HMG-CoA reductase-associated fungal products, such as farnesol, which is a virulence factor of *C. albicans*. Moreover, statins may also cause the direct apoptosis of fungal cells by inhibiting protein isoprenylation. Statins inhibit the synthesis of different prenyl groups in fungi, which are important for the attachment of lipids to the heterotrimeric G-proteins gamma subunit, guanosine triphosphate(GTP)-binding protein (Ras) and Ras-like proteins. Statins may also inhibit G-protein actions and Ras or Ras-like signaling, which are vital for fungal proliferation and differentiation.[21] In summary, it is likely that more than a single mechanism may be acting simultaneously to inhibit fungal activity.

According to the current results, the duration of statin therapy did not seem to have a significant effect on oral *Candida* carriage. It happened that the majority of the study subjects (84%) were on a 20 mg daily dose and the other 16% were administered other doses, which rendered any statistical attempt to explore the relationship between *Candida* colonization and the statin dose inappropriate. This unavoidable shortcoming was a stumbling block in the way of studying the effect of different statin types and doses on oral *Candida* colonization, which could be overcome in future studies. Nevertheless, laboratory studies have shown simvastatin and atorvastatin to have a superior inhibitory effect on *C. albicans* over other statins.[6,10,16] At the time of the present study, 84% of the HLS group were on either simvastatin or atorvastatin.

While reviewing the literature, it was observed that not all *Candida* species were equally susceptible to the statin inhibitory effect, but *C. albicans* was the most sensitive.[22] This may explain the wide discrepancy in the prevalence of *C. albicans* between the HLS group (18%) and the HLNS group (30%) observed in the present study. It would be valuable for this observation to undergo further investigation.

Whether the achievable serum levels of therapeutic doses of statins are inhibitory to fungal activity is still a matter of debate. Several studies have shown that high concentrations of statins, well above the maximum achievable serum level in humans in therapeutic doses, were needed to induce an antifungal effect.[5,19] On the other hand, a study by Nogueira Brilhante et al. demonstrated that the minimum inhibitory concentration (MIC) of simvastatin against *C. albicans* was similar to the serum levels of the drug when administered to control blood cholesterol.[6] It is tempting to speculate about the presence of genotyping differences in susceptibility to statins among *Candida* species. Further clinical studies are needed to investigate whether the therapeutic doses of statins can produce salivary levels of the drug that are sufficient to inhibit fungal activity, and to elucidate whether statins combined with antifungal agents can aid in controlling recalcitrant oral candidiasis. Research has shown that when statins are combined with a number of clinically used antifungal agents, they act synergistically and result in substantial decreases in the the therapeutic doses of antifungal agents.[5,6,10] According to these results, whether statins can be applied in the current clinical practice to treat oral fungal infections needs more investigation.

Limitations

There are limitations to this study. The sample size was relatively small (50 statin users and 50 non-users). Due to the strict selection criteria that were adopted, it was difficult to recruit more subjects within the study time frame. It is possible that future larger-scale studies will help elucidate the effect of the statin dose or the duration of therapy on oral fungi, which was not possible in this study. The largest proportion of patients (84%) was on a 20 mg daily statin dose, which rendered exploring the relationship between oral *Candida* colonization and different statin doses not feasible.

Conclusions

Within the limitations of this study, the therapeutic dose of statins was associated with a reduction in the prevalence and count of oral *Candida*. Larger-scale studies are needed to define the relationship between this antifungal effect and the dose, type and duration of statin therapy, and its efficacy in controlling clinical oral candidal infection.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Institutional Review Board at KAUH (ref. No. 1/116/2018) in compliance with the Declaration of Helsinki and all participants provided written informed consent prior to the investigation.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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