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Clinical Trial

Effectiveness of garlic oil in the treatment of arsenical palmar keratosis

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Abstract

Thousands of Bangladeshi are suffering from arsenic-induced keratosis affecting palms and soles. Palmar keratosis, particularly in young female, affects the socioeconomic condition. Till today there is no effective treatment. Twenty patients of mild to moderate degree of arsenical palmar keratosis were treated with garlic oil in soft capsule (10 mg) daily orally for 12 weeks. Similar treatment was given to 17 arsenic exposed controls and 15 healthy volunteers for comparison. Effectiveness of treatment was evaluated by change in the clinical score of palmar keratosis and amount of total arsenic in nail after 12 weeks. The mean (± SD) clinical scoring of patients before treatment was 102.8 ± 19.0. It was reduced to 36.0 ± 8.7 after completion of treatment (65% reduction). The mean amounts of total arsenic in nail of patients and arsenic exposed controls were 13 to 14-fold higher in comparison to healthy volunteers. Treatment with garlic oil reduced about 50% of the total arsenic accumulated in nails. Common adverse effects were garlic smell and gastric irritation. However, there was no drop out case due to these adverse effects. Oral administration of garlic oil improves symptom of arsenical palmar keratosis with reduction in body arsenic load.

Introduction

At present about 77 million Bangladeshi (half of the total population) are consuming high concentration of arsenic through drinking water and food (Argos et al., 2010; Khan et al., 2007). Chronic consumption of arsenic leads to skin manifestations (melanosis, keratosis, Bowen's disease, squamous cell carcinoma), hypertension, diabetes mellitus, etc (Misbahuddin et al., 2011). Among the skin manifestations, melanosis is mainly present in the unexposed part of the chest, back, inner side of the thigh. It does not create any social problem. On the other hand, keratosis occurs in palms or soles or both. Both hands or soles are equally affected. The presence of keratosis in palms of unmarried girl is a social problem. It needs to be treated. Still now there is no effective treatment of arsenical keratosis. Topical application of salicylic acid (Islam et al., 2007) or propylene glycol (Dina and Misbahuddin, 2010) cures palmar keratosis by their soothing effect. Stoppage of drug application often recurs the symptom.

Our foodstuffs including stable food, rice, are also contaminated with high concentration of arsenic. However, some of our foodstuffs have the capability to remove arsenic from the body. These include corn (Chowdhury et al., 2009), spinach (Umar, 2007), *Amaranthus esculentus* leaves (locally called data shak) and spirulina (Misbahuddin et al., 2006). Our people use garlic (*Allium sativum* L.) in their daily diet as spice which has, surprisingly, less amount of arsenic (Khan et al., 2007). Co-administration of garlic extract (2 mg/mL)

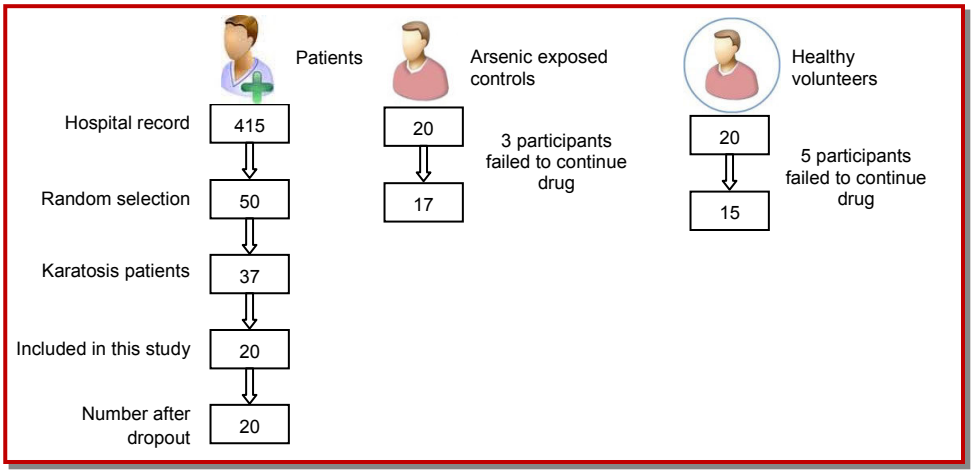

Figure 1: Flow-chart of subject selection

with 10 µM $NaAsO_2$ attenuated arsenite-induced cytotoxicity, reduced intracellular reactive oxygen species (ROS) level in human malignant melanoma cells (A375), human keratinocyte cells (HaCaT) and in cultured human normal dermal fibroblast cells (Chowdhury et al., 2008).

As a supplement garlic is available in drug store as garlic essential oil, garlic oil macerate, garlic powder, and garlic extract. In the present study, we examined whether oral administration of garlic oil is effective in the removal of arsenic from the body as well as clinical improvement of arsenical palmar keratosis.

Materials and Methods

The study was conducted in an arsenic affected area (District: Comilla, Upazilla: Lakshum), 115 km away from the capital city Dhaka. The total area of Lakshum is 22 sq km with a population of 661,800. People of this Upazilla were using 40,170 shallow tube wells for drinking, cooking and land irrigation purposes of which 31,904 tube wells (about 80%) were contaminated with high concentration of arsenic.

There were 415 patients registered in the record book of the Lakshum Upazilla Health Complex of which 50 patients were selected randomly by online random number generator (http://www.psychicscience.org/random.aspx) (Figure 1). Among them, 37 patients were found to suffer from mild to moderate degree of palmar keratosis. The clinical diagnosis of palmar keratosis was confirmed by a dermatologist. Final diagnosis of arsenic palmar keratosis was confirmed by the presence of keratosis in palms, high concentration of arsenic in drinking water (>50 ppb) and nail (>1 µg/g of nail). After counseling of each patient, only 23 agreed to participate in this study. For simplicity, the number of patients were limited to 20 (round figure). Same number (20) of arsenic exposed controls (having no signs and symptoms of melanosis and/or keratosis) were selected from the family members of those patients. Up to two family members from each patient was included in this study. Inclusion of family members depended on their acceptance to participate. Twenty health volunteers were also selected from the same area having exposure to arsenic safe drinking water (<50 ppb) and no sign/symptom of arsenicosis. After completion of this study (12 weeks), the total number of drop out cases were eight of which three from arsenic exposed controls group and five from healthy volunteers group. These were due to failure in regular intake of garlic oil. There were no drop outs from the patients group.

Among the arsenical keratosis patients (four males; 16 females), six were in mild degree and 14 in moderate degree. There were two males and 15 females from arsenic exposed controls. In case of healthy volunteers only one was male and 14 were females (Table I). Age ranges were 21 to 60 years.

Hardening and roughness or gritty feeling of the skin of palm or just palpable or just visible pinhead like keratosis, scatteredly affecting the palm was identified as mild keratosis (Dina and Misbahuddin, 2010). Moderate keratoses were palpable and visible keratosis affecting palm. Severe keratoses were cauliflower like keratosis densely or extensively distributed in whole palm. The scoring of palmar keraosis was done based on the number and size of the keratotic lesions in both palms. If the lesion size was less than 1 mm (as measured by slide caliper), the score was one. If it was in between 1 to 2 mm, the score is 2. If it was more than 2, the score was 3.

The study protocol was approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University. Written informed consent of each participant was taken after explaining the purpose of the study. Patients with only melanosis, severe degree of keratosis, absence of keratosis in palm (present in sole only), age below 20 years and above 60 years,

Table I

Characteristics of participants

Characteristics	Arsenicosis patients	Arsenic exposure controls	Healthy volunteers
n	20	17	15
Age (Years); range	41 ± 12 (22 – 60)	39 ± 13 (21 – 60)	35 ± 13 (21 – 60)
Sex			
Male	4	2	1
Female	16	15	14
Amount of arsenic in water (µg/L)	259.9 ± 59.8	259.4 ± 57.8	31.7 ± 6.6
Duration of arsenic exposure (Years)	11.0 ± 6.0	8.7 ± 4.0	-
Duration of symptoms (Years)	5.6 ± 4.0	-	-

pregnancy, nursing mother, or patients with liver or kidney impairment were excluded from this study.

All the participants were treated at a Temporary Arsenic Camp (house of a patient) from October 2011 to July 2012. All the participants were not treated at the same time. Each participant visited the Arsenic Camp once every two weeks (10 AM to 12 noon) to collect the drug, checking the adherence (by counting the capsules remaining in the bottle), receiving sheets for monitoring adherence and adverse effects, and distributing new sheets for monitoring adherence and adverse effects. In case of patients, in addition, scoring of palmar keratosis were done and recorded. Participants tick marked the sheets for intake of drug as well as adverse effect(s) daily by themselves. The authors sometimes monitor this activity at random by direct contact with the participant by mobile phone. Patients of arsenical palmar keratosis were followed-up every 2 weeks for 8 weeks after completion of treatment.

Fifty milliliters of drinking water was collected once from each participant before starting the study for the estimation of total arsenic. Water sample was collected from the tube well in a polypropylene container containing one drop of concentrated nitric acid. The sample was transported to the laboratory on the same day of collection for estimation of total arsenic. About one gram of finger and toe nails from all the participants were collected in a small polyvenyl bag twice (before and two weeks after completion of treatment). Two milliliter of blood was collected twice (before and two weeks after completion of treatment) from each participant for examining any adverse effect of garlic oil on liver.

Soft capsule of garlic oil (10 mg) was prepared by a local pharmaceutical company (Drug International Limited). Each capsule was coated with methylacrylate-ethylacrylate copolymer in order to inhibit the release it within the stomach and thereby reduce the gastric upset. The garlic oil present within the soft capsule contain allicin (50%) which was confirmed by gas chromatography. Each participant was advised to swallow one capsule daily (morning and evening) with 150 mL of water. We could not ensure the supply of arsenic safe drinking water for swallowing the capsule, drinking and cooking purpose as because 80% of the total tube wells were contaminated with arsenic.

Nails were washed once with acetone for 2 min and then dried naturally before weighed and estimation of total arsenic by SDDC method. The amounts of total arsenic in water and nail were estimated by SDDC method (Misbahuddin et al., 2006). In SDDC method (in brief), samples were digested by four acids (hydrochloric acid, nitric acid, sulfuric acid and perchloric acid) and arsine generated before estimation of red color complex using spectrophotometer at 525 nm. Two percent of the samples for the estimation of arsenic in water was verified by a second laboratory. Blood was used to collect serum for the estimation of transaminases level by Kit method.

One way analysis of variance (ANOVA) was employed for comparisons among groups and paired Student's 't' test was used for comparisons before and after treatment with garlic. The data were expressed as mean ± SD.

Results

Patients and arsenic exposed controls were using drinking water from the same source of tube well having high concentration of arsenic (259.9 ± 59.8 µg/L; Table I). However, the duration of arsenic exposure was different (patients vs arsenic exposed controls: 11.0 ± 6.0 vs 8.7 ± 4.0 years). The duration of symptoms of palmar keratosis in patients was 5.6 ± 4.0 years.

The mean clinical scoring regarding palmar keratosis (n = 20) before treatment was 102.8 ± 19.0 (Figure 2). It was reduced to 36.0 ± 8.7 after treatment (i.e., 64.7% reduction).

The amount of total arsenic in nail was several fold higher in patients (4.3 ± 1.1 µg/g of nail) and arsenic exposed controls (4.0 ± 0.5 µg/g of nail) in comparison to healthy volunteers (0.3 ± 0.3 µg/g of nail; Table II). Treatment with garlic oil for 12 weeks reduced more than 50% of the total accumulated arsenic. Garlic oil was equally effective in both patients and arsenic exposed controls.

All the participants except one complaint of garlic smell (Table III) during the whole treatment period. More than 50% of them had gastric irritation (abdominal cramp, indigestion). Other adverse effects were vertigo (20-30%), diarrhea (0-10%) and constipation (0-7%). There was no drop out cases or no withdrawals due to adverse effects.

Treatment with garlic oil for 12 weeks significantly redu -ced the liver enzymes (transaminases) level indicating that chronic treatment did not worsen the liver function (Table IV).

Each patient was followed-up for another 8 weeks but there was no recurrence of symptoms.

Discussion

Among the patients, 80% were female. These did not mean that females were mainly affected. Previous studies show that males were mainly affected (Watanabe et al., 2001; Sinha et al., 2003). However neither a male or female preponderance with respect to arsenicosis was detected in the records of the Upazilla Health complex.

Greater number of female patients in this study was due to their free time to attend the Temporary Arsenic Camp in order to receive medicines at regular interval (2 weeks). On the other hand, male patients had inadequate time to attend the camp instead they were busy with their job (main source of earning). This conception is reflected by the number of males in other groups of this study.

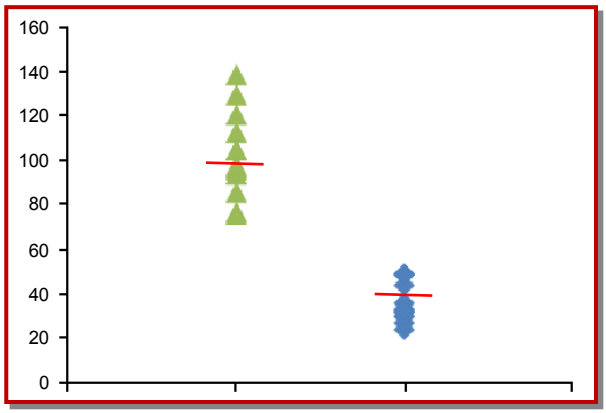

Figure 2: Scoring of both palms of palmar keratosis of individual patient (n = 20) before (closed triangle) and after (closed diamond) treatment with garlic oil. Some of the data overlaps each other. Horizontal red line indicates the mean value

Patients and arsenic exposed controls were using drinking water from the same source of tube well having high concentration of arsenic. However, the duration of arsenic exposure were not same (patients vs arsenic exposed controls: 11.0 ± 6.0 vs 8.7 ± 4.0 years). This variation may be due to migration of people or change of drinking water source.

The mean clinical scoring of patients with respect to palmar keratosis was reduced by 65% following treatment. This finding was supported by the amount of arsenic in nail. It was reduced to about 50% following treatment with garlic oil for 12 weeks. Body arsenic level of arsenicosis patient was also reduced by

Table II

Removal of arsenic from nail after 12 weeks of treatment with garlic oil

Groups	n	Amount of arsenic (µg/g of nail)		Percent reduction	p value
		Before treatment	After treatment		
Arsenicosis patients	20	4.3 ± 1.1	1.9 ± 0.5	55.8	0.000
Arsenic exposure controls	17	4.0 ± 0.5	1.9 ± 0.6	52.5	0.000
Healthy volunteers	15	0.3 ± 0.3	0.2 ± 0.2	33.3	0.021

p value between the groups (using one-way ANOVA) before treatment is 0.000; after treatment is 0.000; Before treatment: P vs AE, p= 0.240; P vs HV, p= 0.000; AE vs HV, p= 0.000; After treatment: P vs AE, p= 0.802; P vs HV, p= 0.000; AE vs HV, p= 0.000

Table III

Number of subjects (with percentage) with adverse effects after 12 weeks treatments with garlic oil

Characteristics	Arsenicosis patients (n = 20)	Arsenic exposure controls (n = 17)	Healthy volunteers (n = 15)
Garlic smell	19	17	15
Gastric irritation	12	10	8
Vertigo	6	4	3
Diarrhea	2	1	0
Constipation	0	0	1

Table IV

Mean serum transaminases levels of participants before and after treatment with garlic

	Patients			Arsenic exposed controls			Healthy volunteers			p value (before treatment)		p value (after treatment)	
	Treatment			Treatment			Treatment						
	Before	After	p value	Before	After	p value	Before	After	p value	P vs H	P vs A	P vs H	P vs A
Plasma ALT (U/L)	59.0 (15.9)	46.0 (7.0)	0.000	45.0 (4.6)	40.0 (3.4)	0.001	33.0 (4.1)	30.0 (3.3)	0.095	0.000	0.000	0.000	0.002
Plasma AST (U/L)	61.0 (12.3)	46.0 (6.8)	0.000	44.0 (4.4)	38.0 (2.7)	0.000	36.0 (3.3)	34.0 (2.1)	0.019	0.000	0.000	0.000	0.000

Data within the parenthesis is SD; P vs H means patients vs healthy volunteers, P vs A means patients vs arsenic exposed controls; p value <0.05 is considered significant

spirulina (Misbahuddin et al., 2006). Now the question is how garlic oil reduces body arsenic load. The exact mechanism of action is not known. Arsenic binds with sulfhydryl groups in cells or enzymes (Winski and Carter, 1995). Garlic contains a lot of sulfur which has the high affinity to bind with sulfhydryl group (Gupta and Porter, 2001) by displacing arsenic. Thus the body arsenic load may be reduced by this way.

Garlic smell is a common complaint which is due to the presence of allicin present within the garlic. Half of the total participants had gastric irritation. We usually take garlic as spices. Raw garlic is usually avoided due to its gastric upset effect, which is due to the presence of high sulfur content. In this study, we have used garlic oil. Like raw garlic, garlic oil produces garlic smell, gastric upset. However, the degree of adverse effects are low and tolerable. There was no drop case due to adverse effects.

Garlic is safe when used in cooking. However, excessive consumption can cause strong breath and body odors (Amagase, 2006). It occasionally causes allergies that can range from mild irritation to potentially life-threatening problems. Ingestion of fresh garlic bulbs, extracts, or oil on an empty stomach may cause heartburn, nausea, vomiting, and diarrhea. Chemical burns, contact dermatitis, and bronchial asthma can occur when garlic is applied to the skin.

Garlic has been used for thousands of years for culinary, medicinal and spiritual purposes. It is effective in mild hypertension (lowers blood pressure by 5-7%), inhibits platelet aggregation, enhances fibrinolytic activity, reducing clots on damaged endothelium. *In vitro* study suggests its antiviral and antibacterial effects, but not established in clinical trial. Garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids, and minerals such as selenium (Kemper, 2000).

Garlic was reported in the ancient Egyptians medical text *Codex Ebers* (ca. 1550 BC) as an important medicine (Banerjee and Maulik, 2002). It was fed to the athletes for increasing stamina during the earliest Olympics in Greece (Lawson, 1998). In ancient Chinese medicine, it was prescribed in diarrhea and worm infestation (Woodward, 1996). Three ancient medical traditions in India i.e., Tibbi, Unani and Ayurveda, made extensive use of garlic as a central part of the healing efficacy of plants (Moyers, 1996). In *Charaka-Samhita (*leading Indian ancient medical textbook) recommends garlic for the treatment of heart disease and arthritis for over many centuries. In another ancient Indian medical textbook, *Bower Manuscript* (~300 AD), garlic was used for fatigue, parasitic disease, digestive disorder and leprosy.

The aqueous garlic (*Allium sativum* L.) extract (2 mg/mL) co-administered with 10 µM $NaAsO_2$ attenuated arsenite-induced cytotoxicity, reduced intracellular reactive oxygen species (ROS) level in human malignant melanoma cells (A375), human keratinocyte cells (HaCaT) and in cultured human normal dermal fibroblast cells (Chowdhury et al., 2008).

The main constituent of garlic oil used (50%) in this study was allicin. Allicin kills microrganisms but its use as antibiotic is not acceptable because of its extreme instability and toxicity. Allicin decomposes to diallyl disulfide (DADS) (66%), diallyl sulfide (DAS) (14%), diallyl trisulfide (9%), and sulfur dioxide at 20°C for 20 hours (Brodnitz, 1991). It easily reacts with amino acids and proteins, creating an -SH group. Allicin binds to protein and fatty acids in the plasma membrane of the lumen, is thus trapped before absorption, and cannot circulate in the blood (Freeman and Kodera, 1995). Therefore, allicin acts either at the gut level or other constituents of garlic oil is effective in the treatment of arsenicosis.

Large dose of garlic reduces the risk of stomach cancer (Li et al., 2004). Topical application of ajoene, an organosulfur compound of garlic, can reduce basal cell carcinoma tumor size, mainly by inducing the mitochondria-dependent route of apoptosis (Tilli et al., 2003). Increased garlic consumption is associated with a statistically significant reduction in breast cancer risk (Challier et al., 1998). Protective effects from garlic may arise from its ability to block the formation of cancer-causing substances (Shenoy and Choughuley, 1992), halt the activation of cancer-causing substances (Milner

2001), enhance DNA repair (L'vova and Zasukhina, 2002), reduce cell proliferation, or induce cell death. Studies are required to see the effects of garlic oil on the treatment of arsenic-induced cancer.

In conclusion, this study shows that garlic oil is effective in the treatment of arsenical palmar keratosis based on clinical improvement of symptoms and reducing body arsenic load. This is the first study showing the effectiveness of garlic oil in the treatment of arsenical palmar keratosis.

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