



Green vegetable juice as a potential source of human fascioliasis in Korea

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ABSTRACT

Fascioliasis, a food-borne helminthiasis, is primarily a disease of cattle and sheep that occasionally occurs in humans. The aquatic perennial herb water dropwort (*Oenanthe javanica*) has been identified as the primary source of human infections in Korea. Recently, a cluster of patients who had not ingested water dropwort, but had the use of a green vegetable delivery service in common was diagnosed with fascioliasis. Our study aimed to identify the association between the green vegetable juice delivery service and the occurrence of human fascioliasis.

Patients with liver abscesses and eosinophilia were enrolled in this study. They were categorized into fascioliasis or non-fascioliasis groups according to serological test results, clinical manifestations, and computed tomography or magnetic resonance imaging findings. Patients were classified into the fascioliasis group when ova or the adult worms of the *Fasciola* species were detected or serological tests were positive, with compatible clinical and radiological findings.

We included 30 patients in this study; 15 were assigned to the fascioliasis group and the remaining 15 to the non-fascioliasis group. The proportion of patients who utilized the juice delivery service was significantly higher in the fascioliasis group than in the non-fascioliasis group (53.3% vs. 0%, $P < 0.01$). Most of the other patients in the fascioliasis group had known risk factors and the intake of water dropwort or other raw vegetables.

This study suggests that human fascioliasis could be transmitted by green vegetable juice produced and delivered by modern industrial systems. Further research on the product, industry and farm-level situations is required to validate these findings.

1. Introduction

Although the burden of foodborne helminthiasis has been reduced in industrialized countries, foodborne parasites continue to have a notable presence in Korea. A nationwide anti-parasitic control program was implemented between 1969 and 1995, reducing the whole helminth egg-positivity rate from 84.3% in 1971 to 2.6% in 2012 [1]. However, according to the 8th National Surveillance on the Prevalence of Intestinal Parasitic Infections in Korea, food-borne parasitic infections have continued to occur, unlike soil-transmitted helminths, which are only seldomly reported [2].

Concurrently the demand for fresh organic and natural products has

increased, and the green vegetable juice delivery industry has subsequently grown in popularity. The industry manufactures fresh juice by squeezing produce in the factory and delivering the juice to the home or office of the consumer in a short time. Several companies provide accessible daily delivery services of green vegetable juice throughout Korea [3]. However, contaminated raw fruit and vegetables have been increasingly recognized as potential sources of pathogenic microorganisms [4]. Fresh-pressed juices are made from raw, unheated vegetables and thus, have the potential risk of microorganism transmission. *Salmonella* species and *Escherichia coli* O157 have been reported as typical pathogens in contaminated fruit juice [5]. However, food-borne helminthiasis has not been associated with fruit or vegetable juice in

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In 2020, two patients were diagnosed with fascioliasis at Asan Medical Center, a 2700-bed tertiary care hospital in Seoul. Their clinical manifestations were very similar. Both initially complained of mild abdominal discomfort, were afebrile, and showed marked peripheral eosinophilia. Abdominal computed tomography (CT) revealed liver abscesses in the patients. Initially, they were treated with empirical antibiotics and anti-helminthic drugs albendazole and praziquantel without significant improvement. Further examination revealed the ova of *Fasciola* species in the bile of one patient. An endoscopic procedure retrieved adult *Fasciola* species from the common bile duct of the second patient. Following the diagnosis, the probable exposure was thoroughly investigated. Interestingly, both patients were found to have used the green vegetable juice delivery service of the same company for several months. They regularly consumed green juice comprised of mixed vegetables, including water dropwort (*Oenanthe javanica*), an aquatic perennial herb notorious as a source of fascioliasis in Korea [6]. Therefore, we performed a prospective study to identify the association between the utilization of green vegetable juice delivery services and human fascioliasis.

2. Methods

2.1. Study population

This study was performed at Asan Medical Center and Dongguk University Ilsan Hospital in South Korea. Adult patients (≥ 18 years old) with peripheral eosinophilia (> 500 eosinophils per μL) and liver abscesses were enrolled in the study from January 2021 to July 2022. Demographic characteristics were identified, and food habits and the use of green vegetable juice delivery services were recorded. Antibodies to other common food-borne helminths in Korea, *Clonorchis sinensis*, *Paragonimus westermani*, and *Toxocara* species, were also reviewed. Characteristic findings in computed tomography (CT) or magnetic resonance imaging (MRI) were also recorded. Patients diagnosed with *P. vivax malaria* were invited to participate in the study as a negative control group for the laboratory tests.

This study was approved by the ethical committee of Asan Medical Center (2021–0492) and Dongguk University Ilsan Hospital (2021–12-025). Informed consent was acquired from all participants.

2.2. Validation of the antibody detection method

Given the lack of Ministry of Food and Drug Safety (MFDS)-approved commercial kits to measure antibodies to the *Fasciola* species, we performed a validation test of a commercially available kit (Creative Diagnostics, Shirley, NY, USA). This kit measures antibodies to *Fasciola hepatica* by the enzyme-linked immunosorbent assay (ELISA) method. Positive serum samples were obtained from three patients, including two who had parasitological confirmation of *Fasciola* infections. The other patient was later diagnosed with *Fasciola*. She had a liver abscess that did not respond to antibiotics, and parasite ova were confirmed in the liver abscess. Negative serum samples were obtained from patients with *P. vivax* infections. *P. vivax*, malaria-causing protozoa, has complex genetic architecture distinct from other helminthiases, minimizing cross-reactions when measuring antibodies. Using the test-provided cut-off control as a reference, the relative ratio of the optical density (OD) values between the samples and controls were compared.

2.3. Subgroup definition

Patients with parasitological confirmation of fascioliasis, defined by the detection of ova or adult worms during the evaluation, were classified as having definite fascioliasis. Patients were considered to have probable fascioliasis when they had the following characteristics: positive serological tests for *F. hepatica*, peripheral eosinophilia, and

compatible CT or MRI findings of liver abscesses or bile duct dilatation. Patients who did not meet the above conditions were assigned to the non-fascioliasis group.

2.4. Statistical analysis

Statistical analyses were performed using SPSS version 23.0 (IBM Co., Armonk, NY, USA). The two groups were compared using the Student's *t*-test, chi-squared test, or Fisher's exact test as appropriate. All tests of significance were two-tailed, and differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Diagnostic performance of ELISA

Three positive samples and 10 negative samples were used in the validation study. Duplicate tests were performed for all the samples, and the mean values were used. The mean OD ratio of the positive and negative samples was 4.79 and 0.46, respectively, which was statistically significant ($P < 0.01$) (Fig. 1a). Although the sample size was small, the test kit effectively differentiated between positive and negative samples.

3.2. Study population

A total of 30 patients participated in the study. The mean age of all patients was 55.1 years old, 22 (73.3%) of whom were male. Considering the clinical manifestations, radiographic findings, and ELISA results, 15 patients were assigned to the fascioliasis group and 15 to the non-fascioliasis group. Among the fascioliasis group, three patients had definite fascioliasis, and 12 had probable fascioliasis. The clinical and radiographic findings of the two groups are summarized in Table 1, and the ELISA results are presented in Fig. 1b.

3.3. Comparison of fascioliasis and non-fascioliasis group

The proportion of patients who used the green vegetable juice delivery service was significantly higher in the fascioliasis group (53.3% vs. 0%, $P < 0.01$). The duration of green vegetable juice delivery service in eight patients who utilized the service ranged from 7 months to 15 years. Five patients in the fascioliasis group who did not use the green vegetable juice delivery service reported the frequent consumption of water dropwort salad. The other patient reported the consumption of raw vegetables in Hanoi, Vietnam, where he had resided for six months. A similar proportion of participants consumed raw water dropwort salad in both the fascioliasis and non-fascioliasis groups (40.0% vs. 33.3%, respectively, $P = 0.71$). Two patients reported that they had drunk homemade vegetable juice. However, they were in the non-fascioliasis group, suggesting that the homemade vegetable juice was less likely to be a source of fascioliasis. Other dietary habits regarding the confirmed sources of foodborne helminthiasis, such as the consumption of raw meat, freshwater fish, or soy sauce-marinated crab, did not differ between the two groups.

All 15 patients classified into the fascioliasis group had higher peripheral eosinophil levels than the non-fascioliasis group. One or two liver abscesses were detected, of which the largest diameter was approximately 5.5 cm, significantly larger than that of the non-fascioliasis group. Antibodies to *C. sinensis* and *P. westermani* were positive in 35.7% and 57.1%, respectively, of the patients in the fascioliasis group. However, only 6.7% and 20.0% of patients had possible exposure to *C. sinensis* (raw freshwater fish) and *P. westermani* (soy sauce-marinated crab), respectively.

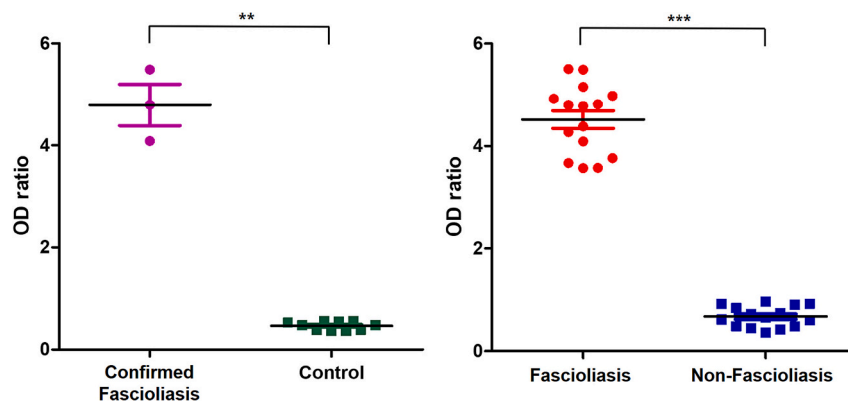


Fig. 1. (a) Distribution of OD ratio obtained for the ELISA for confirmed fascioliasis cases and control sera. (b) Distribution of OD ratio obtained for the ELISA of the fascioliasis and non-fascioliasis groups. Lines represent median values and interquartile ranges.

** Indicates P -values of <0.01 and *** indicates P -values of <0.001 .

Table 1

Baseline clinical characteristics of the fascioliasis and non-fascioliasis groups.

Characteristics	Total	Fascioliasis (n = 15)	Non-fascioliasis (n = 15)	P -value
Mean age (mean \pm SD), years	55.1 \pm 14.5	48.0 \pm 12.3	62.1 \pm 13.2	< 0.01
Male sex	22 (73.3)	12 (80.0)	10 (66.7)	0.68
Eating habits				
Green vegetable juice	8 (26.7)	8 (53.3)	0 (0)	< 0.01
Water dropwort salad	11 (36.7)	6 (40.0)	5 (33.3)	0.71
Raw beef liver	7 (23.3)	1 (6.7)	6 (40.0)	0.08
Raw meat	7 (23.3)	1 (6.7)	6 (40.0)	0.08
Raw freshwater fish	3 (10.0)	1 (6.7)	2 (13.3)	> 0.99
Soy sauce-marinated crab	6 (20.0)	3 (20.0)	3 (20.0)	> 0.99
Positive antibody test*				
<i>Clonorchis sinensis</i>	8/29 (27.6)	5/14 (35.7)	3/15 (20.0)	0.43
<i>Paragonimus westermani</i>	10/29 (34.5)	8/14 (57.1)	2/15 (13.3)	0.02
<i>Toxocara</i> spp.	13/25 (52.0)	3/13 (23.1)	10/12 (83.3)	< 0.01
Peripheral eosinophil count (mean \pm SD), per mm ³	1847.6 \pm 1268.1	2477.4 \pm 1396.6	1259.9 \pm 798.9	0.03
Liver abscess				
Number of lesions (mean \pm SD)	1.8 \pm 1.6	1.1 \pm 0.4	2.3 \pm 2.1	0.047
Largest diameter (cm)				
< 3	13/29 (44.8)	1/14 (7.1)	12/15 (80.0)	
≥ 3	16/29 (55.2)	13/14 (92.9)	3/15 (20.0)	< 0.01

Data are given as mean \pm SD or as number (percentage).

* All three antibodies were not measurable in all patients.

4. Discussion

In this study, we aimed to identify the association between green vegetable juice consumption and human fascioliasis. Half of the patients in the fascioliasis group had used a green vegetable juice delivery service, whereas none had in the non-fascioliasis group, which was a significant difference. In contrast, the intake of water dropwort salad, known to be associated with a risk for fascioliasis did not differ between the two groups. Therefore, our study results suggest that industrialized green juice delivery services could be a new source of human fascioliasis.

Fascioliasis is a zoonotic disease that has existed in humans for several hundred years in Korea [7]. Cattle are considered a major reservoir in Korea, and humans have become infected by ingesting water

plants contaminated with the metacercaria of the *Fasciola* species. Water dropwort, an ingredient in soups and vegetable salads, has long been considered a major source of human *Fasciola* infections in Korea. One study revealed that 0.4% of water dropwort in farms was contaminated with *F. hepatica* metacercariae [8]. It is speculated that the metacercariae on the surface of water dropwort might be transferred to green vegetable juice in a viable state during the commercial juice-making process. Furthermore, water dropwort is often consumed more frequently and in larger quantities in juice than in salads, thus revealing green vegetable juice as a more prominent source of infection.

The clinical manifestations of patients in the fascioliasis group were generally consistent with the early phase of fascioliasis [9,10]. After ingesting metacercaria, the larva penetrates the intestinal wall, moves into the peritoneal cavity, and invades the peripheral part of the liver. Thus, liver abscesses and peripheral eosinophilia are the dominant clinical findings during this phase. During the late and chronic phases, the developed worm appears in the bile duct, causing intermittent obstruction or inflammation [11,12].

Notably, 35.7% and 57.1% of the patients in the fascioliasis group tested positive for *C. sinensis* and *P. westermani* antibodies, respectively. These results could have been due to cross-reactions with *F. hepatica*, given that more than half of the patients lacked exposure to known risk factors, although the potential for co-infection could not be excluded. This antibody cross-reactivity among foodborne helminths could also confuse the diagnosis of fascioliasis.

Among the 15 patients in the non-fascioliasis group, 83.3% had positive serological results for *Toxocara* species, and 40% had a history of raw beef liver or meat consumption. Toxocariasis is usually transmitted by ingesting the ova of *Toxocara* species, and the association between the habitual ingestion of raw beef meat and toxocariasis has been consistently reported in Korea [13,14]. We considered that many patients in the non-fascioliasis group had toxocariasis and thus, were treated empirically with antibiotics and albendazole. The follow-up CT findings revealed improvements.

Our study had several limitations. The most important limitation is that we could not present direct evidence that the metacercariae of *Fasciola* species existed in the commercially available green juice. We conducted real-time polymerase chain reaction (PCR) for *F. hepatica* and *F. gigantica* genes with 100 bottles of the green juice that most of the patients used. However, all the test results were negative. A large quantity of green juice should be examined to prove the causality of human fascioliasis as the consumption of green juice. Second, only a small number of patients were included in the analysis, and the timing and period of exposure depended only on the memory recall of the patients. Additionally, patients were primarily located within the capital area around Seoul where the juice delivery service is popular. Thus, a large-scale study including more patients from various regions of Korea

is urgently needed. Third, there could be other confounding exposures of patients using green vegetable juice delivery services. Health-seeking behaviors may differ between those who use pressed juice delivery services and those who do not. Fourth, potential errors in the diagnosis of fascioliasis may have existed in the probable fascioliasis group. Finally, our study only revealed the causes of human infections with *Fasciola* species. A report suggested that approximately 40% of the cattle raised in Korea were infected with *Fasciola* species until the late 1970s [15]. Currently, 0.54% of cattle [16], 1.5–3.4% of freshwater snails in the farming fields [6,17], and 0.4% of water dropwort [8] are expected to be contaminated with *Fasciola* species. Many unanswered questions remain surrounding the farm-level risk factors for cattle fascioliasis, such as how *Fasciola* species ova could reach water dropwort farms and what food processing methods could prevent human infections.

One health approach to understanding how human infections with *Fasciola* species are maintained in modernized livestock and the fresh juice industry era is urgently needed.

CRedit authorship contribution statement

Sungim Choi: Conceptualization, Writing – original draft. **Sunghee Park:** Conceptualization, Methodology. **Sooji Hong:** Methodology, Resources. **Hyejoo Shin:** Methodology, Resources. **Bong-Kwang Jung:** Conceptualization, Writing – review & editing. **Min Jae Kim:** Conceptualization, Formal analysis, Writing – original draft.

Declaration of Competing Interest

We have no conflicts of interest related to this work.

Data availability

Data will be made available on request.

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