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Improvement of Ritchie Technique by Identifying the Food That Can Be Consumed Pre-analysis

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Abstract: The objective of this study is to identify foods that cause large sediments, which interfere with Ritchie technique. This technique is used routinely in diagnostic clinical laboratories for detection of intestinal parasites in stool specimens. Foods included in the study were classified into six groups; 1- Vegetables and beans, 2- Fruits, nuts and seeds, 3- Meat, fish and poultry, 4- Grains, 5- Milk and dairy food, 6- Spices and miscellaneous. From each of these groups, several foods were investigated by Ritchie technique. In addition, several volunteers were asked to consume specified food, then stool specimens from those volunteers were examined by Ritchie technique. Most of the studied foods resulted in small sediments and large debris plug. Those foods that formed large amount of sediments were identified and listed according to their food group. A list of these foods can be provided for patients to avoid consumption for 2 days before performing Ritchie technique for detection of intestinal parasites.

Key words: Parasites, Intestinal diseases, Laboratory diagnosis, Ritchie technique

INTRODUCTION

Intestinal parasites are considered to be one of the main public health problems around the world especially in tropical and sub tropical countries. It is estimated that some 3.5 billion people are affected, and that 450 million are ill because of these infections (i.e. some 3 billion are asymptomatic carriers) and yearly, some 200,000 deaths are due to intestinal parasitic infections[1].

Several concentration techniques are used routinely in diagnostic clinical laboratories to increase the chance of recovery for diagnostic stages of intestinal parasites in stool specimen. Ritchie sedimentation technique is one of the best concentration techniques used in diagnostic parasitology laboratories using 10% (v/v) formal saline and diethyl ether. All diagnostic stages that are applicable with Ritchie technique will be concentrated at the bottom of the tube. Remaining of some types of the consumed food could precipitate resulting in increased sediment amount, which makes microscopic examination difficult.

The pioneer idea of this study depends on identification of foods that interfere with Ritchie technique.

MATERIALS AND METHODS

The Investigated Food: During this study, investigated foods were classified into six groups as illustrated below (arranged alphabetically in each group):

A) Vegetables and Beans: This group included artichoke, asparagus, aubergine (eggplant), basil, bean sprouts, beetroot, blackeye bean (pea), broad bean, broccoli, brussels sprouts, cabbage- white, cabbage- red, carrots, cauliflower, celery, chickpea, chili peppers, coriander- green, corn- local, corn- sweet, courgette (zucchini), cucumber, cucurbit, florence, garlic, gherkins, ginger- fresh, green beans, greengages, leeks, lentil- brown, lentil- green, lentil- yellow, lettuce, mint, Jew's mallow, mushrooms, okra (lady's finger), olives- black, olive- green, onions, parsley, parsnip, peas, potatoes, popcorns, pumpkins, purslane, radish, shallots, spinach, spring onions, spring greens, sweet pepper, sweet potatoes, tamarind, thyme, tomatoes, turnips, vine leaves, watercress, and white beans.

B) Fruits, Nuts and Seeds: This group included almonds, apples, apricots, avocado, bananas, Brazil nuts, blackcurrants, cashew nuts, cashew fruit, cherries, chestnuts, clementine, coconut, cucurbit seeds, custard

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apple, dates- dried, dates- fresh, figs, grapes- black, grapes- green, grapes- red, grapefruit, guava, hazelnut, kiwi, lemons, limes, longans, loquats, lupin bean, macadamia nuts, mandarin, mangoes, mulberry, oranges, papayas, passion fruit, peaches, peanuts, pears, pineapple, pine nuts, pistachio nuts, plums, pomegranate, prickly pear fruit, raisins, raspberries, sesame seeds, strawberries, sunflower seeds, sultanas, walnuts, water melons, water melons seeds and yellow melons.

C) Meat, Fish and Poultry: This group included beef, camel meat, chicken, crab, duck meat, eggs- boiled, eggs- fried, fishes (salmon, sardine, shark and 4 kinds of local red sea fishes), lamb, liver, lobster, luncheon meat, prawns, rabbit meat, sausages, sheep heart, sheep kidney, sheep tongue and turkey.

D) Grains: This group included basmati rice, biscuits (several kinds), burger bun, cakes (several kinds), Carlos rice, chapatti bread, cornflakes, couscous, Japanese crackers, lasagna, long grain rice, macaroni, macaroni- wholemeal, mamool, muffins, noodles- rice, noodles- plain, noodles- egg, oatmeal, pastries, rusk, long white bread, long wholemeal bread, shami (pita) white bread, shami (pita) wholemeal bread, spaghetti, Afghani bread, Thai crackers and vermicelli.

E) Milk and Dairy Food: This group included cheddar cheese, cheese spread, cream cheese, feta cheese, fromage frais, milk semi-skimmed, milk-skimmed, milk- whole, mozzarella, parmesan, laban, labnah and yoghurt.

F) Spices and Miscellaneous: This group included aniseed black peppers, black seeds (black cumin), camomile, cardamoms, chocolates, cinnamon, cloves, coffee, coriander- ground seeds, cumin, halawa tehenia, honey, hot sauce, ice-cream (several kinds), jam (several kinds), jelly, ketchup, turmeric powder, sweet spice, sumac and tahini.

Stool Specimens from Volunteers: Twenty volunteers were included in this study. Each volunteer was supplied with a list of specified food from the above groups and asked to consume within 48 hours. In addition, each volunteer was provided with four labeled clean specimen containers and collection instructions. Three or Four stool specimens were collected from each volunteer in 12-hr intervals during the 48 hrs. All stool specimens were examined by Ritchie technique.

Preparation of Food for Ritchie Technique: For hard foods, about 10 gm was homogenized in 50 ml of normal saline (0.85%NaCl) by using Braun food processor, while liquid or semi-liquid foods were used directly. For morphological identification, about 1 mg of this preparation was examined directly under light microscope and photographed.

Preparation of Stool Specimens for Ritchie Techniques: About 2 gm from each specimen was emulsified in 15 ml of 10% (v/v) formal-saline and then allowed to stand for 20 minutes before performance of Ritchie technique.

Performance of Ritchie Technique: The prepared food or stool was strained through two layers of gauze into a 15 ml conical centrifuge tube and centrifuged at 2000 rpm for 5 minutes. After that, the sediment was resuspended with 10ml of 10% (v/v) formal-saline and allowed to stand for 5 minutes. 3ml of diethyl ether was added and shaken vigorously for 20 seconds then centrifuged at 2000 rpm for 5 minutes. After centrifugation, four layers formed. A top layer of ether, a debris plug separated in a layer between the ether and formal saline, and the sediment layer in the bottom of the tube.

Macroscopic Examination: For each tested food, the thickness of debris plug and the amount of the sediment were checked macroscopically with naked eye and reported as small or thick (Fig. 1).

Microscopic Examination: 1-2 drops of iodine and/or normal saline were added to the sediments of food and stool specimens and mixed well. Part from each sediment was transferred to a glass microscope slide, covered with a cover glass and scanned under x10 and x40 objectives as required. Appearance of the field under the microscope was correlated to the sediment amount. Type of sedimented foods in stool specimens were identified according to the microscopic appearance and morphology of the direct examined food as mentioned above.

Listing of Interfering Food: All foods that gave thick microscopic appearance due to the large sediment in the bottom of the analysis tube of stool specimens were identified then listed and reported as "should be avoided for two to three days before stool analysis for diagnosis of intestinal parasites by Ritchie technique".

RESULTS AND DISCUSSION

After performing Ritchie technique on all stool specimens and foods mentioned above, the amount of debris plug and the sediment for each specimen was observed. Most foods resulted in small sediment layer and large debris plug (Fig. 1). Table 1 summarizes the food that interfered with Ritchie technique.

Table 1: List of foods recommended to be avoided by the patient for 2 days before stool analysis by Ritchie technique for easier microscopic detection of intestinal parasites.

A) Vegetables and beans	B) Fruits, nuts and seeds	C) Meat, fish and poultry,	D) Grains	E) Spices and miscellaneous
Artichoke	Almonds	*In general*	Biscuits	Black peppers
Basil	Cashew nuts	All	Bread- All	Cardamoms
Beans	Chestnuts	Fishes	Cornflakes	Chocolates
Cabbage	Coconut	and	Couscous	Cinnamon
Chickpeas	Dates	Red Meat	Crackers	Coriander seeds
Corn	Hazelnut		Lasagna and Macaroni	Cummins
Ginger-fresh	Kiwi		Mamool	Sumac
Lentil	Peanuts		Muffins	Tahini
Mint	Pears		Noodles	Turmeric
Popcorn	Pine nuts		Rice	
Potatoes	Pomegranate		Rusk	
Sweet potatoes	Sesame Seeds		Spaghetti	
Turnip	Walnuts		Vermicelli	

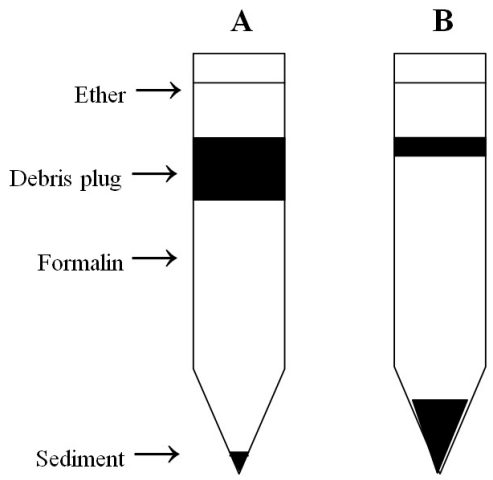

Fig. 1: Appearance of the four layers after pefoming Ritchie techniques, A; with thick debris plug and small sediment layer, B; with small debris plug and thick sediment layer.

Parasites are organisms living in or on another living organism, obtaining from it part or all of their organic nutriment and sometimes can harm their host[2]. These parasites can be protozoa composed of one cell or helminthes. Intestinal parasites are those parasites that inhabit intestine. In addition, diagnostic stages of some non-intestinal parasites can pass through the intestine then appear in stool.

There are several common routine techniques which are applicable for diagnosis of intestinal parasites by using stool specimen.These techniques includ direct smears, thick smears, permanent staining, and concentration techniques by sedimentation and floatation[3].

Concentration sedimentation technique known as Ritchie technique is usually used to increase the recovery chance of the applicable diagnostic stages of the intestinal parasites in the stool by starting with good large amount of stool (2-5 gm) in comparison to direct smears (1-2 mg)[3-6]. Although Ritchie sedimentation technique can not detect trophozoites, it is considered as the best technique used in diagnostic parasitology laboratories for detection of cysts, ova and larvae[4,7].

Generally, 10% formal saline is used in Ritchie technique to kill and preserve diagnostic stages of the parasites. Diethyl ether collects most of unwanted debris in separate layer. All diagnostic stages that are applicable with Ritchie technique will be concentrated at the bottom of the analysis centrifuge tube. Quantitatively, one slide from Ritchie technique is a substitute of about one thousand slides or more from the direct smear technique.

Remaining of some foods precipitate in the bottom of the analysis tube usually causes thick sediment in the bottom of the tube and the diagnostic stages of the parasites cannot be seen easily under the microscope.

The solve for this problem is presented in this study, for the first time, many consumed foods were tested with Ritchie concentration technique. Each food was examined microscopically and identified morphologically before and after addition of iodine. To include any effect of digestive tract acids, enzymes and microbial activity on food, several volunteers were provided with specified types of food to consume for two days. During this time, each volunteer was provided with four clean specimen containers and instruction of stool collection. Stool specimens were processed with Ritchie technique and the formed sediment were examined macroscopically for the thickness (Fig. 1). The precipitated elements were examined microscopically and compared with the morphology of the previously tested foods.

Table 1 illustrates the foods that caused thick sediment interfered with Ritchie technique and recommended to be avoided for two or three days before analysis.

There are several systems of dividing foods into groups. Some countries assign foods into four, five or sometimes up to eight groups. Some included fats and sugars in a separate group while others did not. Food groups according to the United State Department of Agriculture (USDA) were classified into five groups, grains group, vegetables group, fruits group, milk group and meat & beans group. In Canada, foods were classified into four groups by combining vegetables and fruits in one group. Philippines combines milk group and meat group in one group of animal foods.[8,9].

In the present study, spices and miscellaneous were assigned in a separate group, therefore, foods were classified into six groups. The first group included vegetables and beans. Foods in this group that caused thick sediment or thick microscopic field included; artichoke, basil, beans (all tested kinds), chickpeas, corn (all tested kinds), lentil (all tested kinds), mint, cabbage (all tested kinds), ginger-fresh, potatoes, sweet potatoes, turnip, and popcorn. Using iodine, it was clear that most of these foods contain starch cells. In the second group of fruits, nuts and seeds, foods caused thick sedimenet or thick field under microscope include almonds, cashew nuts, chestnuts, coconut, dates (all tested kinds), hazelnut, kiwi, peanuts, pears, pine nuts, pomegranate, sesame seeds and walnuts. The third group included red meats, fishes and poultry. Red meats and fishes in this group were found to cause thick sediment with Ritchie technique. The fourth group included grains of the common rice, bread and cereals in Saudi Arabia. This entire group formed thick sediment with Ritchie technique due to starch cells. The fifth group included the most common milk and dairy products. From Table 1, it is clear that this group has no valauble effect on Ritchie technique. The last group was specified for spices and miscellaneous. It was clear that almost all spices can precipetate and appear as dark particles or granules in the field during microscopic examination for the sedimnt of Ritchie technique.

In conclusion, the present study has shown that the common foods that could interfere with Ritchie technique are those rich with starch, also most of nuts, red meat, fishes and ground spices. The avoidance or minimizing consumption of these foods (Table 1) will result in clear fields and easier microscopic examination of Ritchie technique for diagnosis of intestinal parasites. Finally, our suggestion for other workers to investigate other common foods in their areas, as the food included in this study were obtained from markets in Jeddah, Saudi Arabia.

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