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Brief communication (Original)

Surveillance of marine fish for ciguatera toxin at fish markets in Bangkok, Thailand

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Background: Ciguatera intoxication is a form of food poisoning. It is caused by the consumption of certain warm-water fish that have accumulated ciguatera toxin (CTX) through the marine food chain. Ciguatera fish poisoning (CFP) is a global disease, which although common, has been largely ignored in Thailand. This may be attributed to lack of confirmatory tests and seemingly nonspecific clinical presentations.

Objective: We studied CTX contamination in marine fish sold at Bangkok markets.

Methods: A surveillance of CTX in fish using the Cigua-Check assay has been conducted in Bangkok, the center of seafood marketing in Thailand. Here, there are several types of fish. Some come from domestic fishing and others are imported from Indonesia, Myanmar, or India.

Results: A random survey at three fish markets in Bangkok revealed two samples possibly contaminated with ciguatera toxin from a total 227 fish samples (of 21 fish species). This is the first report of finding CTX contamination in fish meat sold in Bangkok.

Conclusion: It is possible that these CTX-positive fish were caught from other countries. Even though the positivity (0.88%) is lower when compared with studies of other endemic areas (5.0% in Hawaii and Pacific Islands), this finding should raise awareness of the possibility of facing rare CFP intoxication from fish obtained at local Thai markets.

Keywords: Bangkok, ciguatera toxin, marine fish

The most common marine toxin worldwide is from ciguatera. Many types of reef-fish are vulnerable to accumulating ciguatera toxin (CTX). This is particularly true for barracuda, grouper, sea bass, snapper, and amberjack [1]. CTX has been reported in 207 fish species worldwide. A partial list is shown in **Table 1** [2-4]. The endemic areas of ciguatera are oceans in the latitudes between 35° north and south of the equator. It was estimated that at least 25,000 ciguatera fish poisoning (CFP) cases occur each year worldwide. It is the most frequently reported seafood-related disease in Australia, United States, the Caribbean, and Papua New Guinea [5]. The toxin comes initially from dinoflagellate algae, principally the coral reef species *Gambierdiscus toxicus*. Reef disturbances by military activities, fishery activities and tourism, and in addition the effects of global warming, increase the risk of ciguatera outbreaks by increasing benthic substrate for dinoflagellate growth [6]. The destruction of coral reefs allows for greater growth of the algae carrying dinoflagellates [7]. This may also be influenced by the degree of sunlight exposure in the presence of silicates and oxides from nearby land and with algal detritus resulting in the development of peculiar algal turfs *Turbinaria*, *Jania* and *Amphiroa* species. Such growth patterns presumably underlie the spatial and temporal variability of ciguatera outbreaks.

CTX is a lipophilic polyether that can open voltage sensitive Na^+ channels at the neuromuscular junction [8]. It causes hyperexcitability and inhibits synaptic transmission. Ingesting fish meat containing CTX produces an illness very much similar to other food poisoning or gastrointestinal viral, bacterial, or parasitic infections. The majority of patients begin to have signs and symptoms of ciguatera at 6–10 hours after intoxication (range 1–48 hours). Neurological symptoms often manifest early such as headache, numbness, "classic" reversal of temperature perception, ataxia (rare), and muscle paralysis. Some

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sensory symptoms (tingling sensation, cold allodynia, or small fiber polyneuropathy) can persist for weeks, but gastrointestinal symptoms (diarrhea, nausea, and vomiting) and cardiovascular symptoms (hypotension, cardiac arrhythmia) usually resolve within 2–3 days. Generally, the severity of ciguatera intoxication is milder than puffer fish poisoning, but it can cause acute and chronic effects including hallucination, depression, and anxiety [9].

Treatment of CTX poisoning is mainly supportive and symptomatic, such as volume replacement for dehydration. There were studies advocating that mannitol as 0.5–1.0 g/kg intravenous drip in 30–45 minutes within 48–72 hours after onset, would help improve the severity of symptoms. It was believed, that because of high osmolarity, mannitol could reduce neuronal edema and act as scavenger of free radicals [10]. However, in some controlled trials, benefit of mannitol over normal saline infusions could not be demonstrated [11]. Besides, osmotic diuresis may worsens a patient's condition by increasing volume depletion. Until recently, there was no appropriate randomized controlled trial that could prove advantages of mannitol over normal saline.

Ciguatera intoxication has been documented as endemic throughout the subtropical Pacific, tropical Indian Ocean, and the Caribbean Sea [9, 12-13) where *G. toxicus* is located and ciguatoxic fishes have been traditional caught. CTX from fish in the Indian and Pacific oceans have more toxin than those from the Caribbean [14]. The risk mainly comes from the importation of live reef fish caught from high-risk ciguatera regions [15]. Thailand is located in the range of latitude 5° to 20° north of equator and longtitude 97° to 105° east, a ciguatera endemic area, and there has only one reported human case of ciguatoxin poisoning (in 1984). However, the increasing interregional trade of fish, including coral reef fish and other marine products is at least partly because of the deceasing density of fish in Thai waters. The importation of coral fish and other marine products might introduce ciguatera fish poisoning to Thailand. Fish imported to Thailand now comes from several sources, namely Indonesia, Malaysia, Myanmar, Brunei, Cambodia, Vietnam, Yemen, India, and Bangladesh [16]. In this study, marine fish samples and fish fillets were randomly collected from different fish markets in Bangkok and they revealed rare fish flesh contaminated with CTX.

Table 1. Examples of fish associated with ciguatera (adapted from [4])

English name (Species)	Thai name	Distribution
Lined surgeonfish (*Acanthurus linearis*)	ปลาขี้ตังเบ็ดลาย	Indo-Pacific
Bonefish (*Albula vulpes*)	Worldwide in warm seas	Worldwide in warm seas
Gray triggerfish (*Balistes carolinensis*)	-	Atlantic, Gulf of Mexico
Saucereye porgy (*Calamus calamus*)	-	Western Atlantic
Horse-eye jack (*Caranx latus*)	-	Atlantic
Whitetip shark (*Carcharhinus longimanus*)	ปลาฉลามครีบขาว	Worldwide
Humphead wrasse (*Cheilinus undulatus*)	ปลานโปเลียน หรือ ปลานกขุนทองหัวโหนก	Indo-Pacific
Heavy beak parrotfish (*Chlorurs gibbus*)	-	Indo-Pacific
Red grouper (*Epinephelus morio*)	-	Western-Atlantic
Giant moray (*Gymnothorax javanicus*)	ปลาไหลมอเรย์ยักษ์	Indo-Pacific
Hogfish (*Lachnolaimus maximus*)	-	Western Atlantic
Northern red snapper (*Lutjanus campechanus*)	-	Western Atlantic, Gulf of Mexico
Tarpon (*Megalops atlanticus*)	ปลาตาเหลือก	Eastern Atlantic
Narrowhead gray mullet (*Mugil capurri*)	-	East Central Atlantic
Yellowtail snapper (*Ocyurus chrysurus*)	-	Western Atlantic
Spotted coral grouper (*Plectropomus maculatus*)	-	Western Pacific
Blue parrotfish (*Scarus coeruleus*)	-	Western Atlantic
Spanish mackerel (*Scomberomorus maculatus*)	อินทรีบั้ง / เบกา	Western Atlantic
Lesser amberjack (*Seriola fasciata*)	-	Western Atlantic
Great barracuda (*Sphyraena barracuda*)	ปลาน้ำดอกไม้ / ปลาสากเหลือง	Indo-Pacific, Western Atlantic
Chinaman fish (*Symphorus nematophorus*)	-	Western Pacific
Swordfish (*Xiphias gladius*)	ปลากระโทงดาบ	Atlantic, Indo-Pacific, Mediterranean

Materials and methods

Samples collection

A total of 69 fresh fish samples included 20 species of fish (16 of Spanish mackerel, 13 of grouper, 10 of white perch, 4 of red-banded grouper, 3 of large head hair tail, 3 of great barracuda, 3 of Malabar red snapper, 2 of yellow stripe trevally, 2 of common ponyfish, 2 of fourfinger threadfin, 2 of ornate emperor, 1 of false trevally, 1 of humpback grouper, 1 of immaculate puffer, 1 of Indian mackerel, 1 of largescale tonguesole, 1 of purple-spotted bigeye, 1 of guitarfish, 1 of yellow queenfish, and 1 of Chinese pomfret) were collected randomly from three open markets in Bangkok between July and December 2008. These samples were tested for CTX by using the commercial available kit, *Cigua–Check* (ToxiTec, Hawaii, USA). Additionally, five 1-kg packs of silver grunt fillet (lined silver grunt, *Pomadasys hasta*) were randomly collected from the same open markets between March and July 2009. Each of the 30–40 slices in each pack was individually tested for CTX. The total number of slices was 158. These specimens were transported from market to laboratory on ice and then kept frozen at –80°C until tested. In this study, 227 specimens were included.

CTX testing

Each of the 69 fish samples and 158 slices from frozen packs were tested for CTX using a commercially available test kit (Cigua-Check Fish Poisoning test kits) according to the manufacturer' s instructions. Briefly, a small, rice grain-sized piece of meat was cut blood free and handled with clean scissors and forceps. Each sample was put in a vial of methanol. The test stick was then placed into the vial containing clear liquid and fish sample. After incubating the test strip in the vial for 20 min, the test stick was removed and direct air dried for 15 to 20 minutes to remove solvent attached on the surface of the stick. The dry test stick was then placed into the well-mixed blue liquid solution-containing latex immunobeads for 10 min, and then removed and rinsed in distilled water. The test stick was examined against a white background to distinguish any color change on the paper-covered side. Any color change on the test stick indicated that the fish contained CTX. A darker color indicates a higher CTX concentration. Results were scored as follows: negative: no distinct color on the membrane; borderline: light blue color on the membrane; positive: membrane colored. A clear result means there is no CTX contamination and fish is safe to eat. The specimens with borderline or positive results were repeatedly tested in triplicate. If the repeated result turns to negative, it was considered as negative. If all repeated results remained borderline or positive, it was considered as borderline or positive.

A positive control was made by inserting a positive control stick to a well-mixed latex immunobead suspension. After 10 minutes incubation, the stick was rinsed with distilled water. A very faint blue positive color appeared on the stick. The limit of detection of this kit is 0.05 µg/kg fish [17]. The negative control test was conducted in the same manner as used for a fish sample test, but without a fish sample.

Results

Of sixty-nine fish samples examined, no sample tested positive using the Cigux-Check kit. However, two samples of 158 fish-fillet slices from lined silver grunt showed positive results (**Table 2**). These two samples were repeated separately and the results were the same as in the previous test. Unfortunately, these two positive samples were not confirmed by other validated methods such as mouse bioassay, which is widely used and the accepted laboratory test for the detection of CTX levels in fish sample extracts [18] or using the guinea pig atrial assay test. These were not currently available to our laboratory.

Discussion

Recent studies have suggested that the increased prevalence of CFP correlated with global warming and other environmental disturbances [18-20]. Because of the difficulty in identifying marine toxins in the human body or even in fish meat and in shellfish, diagnosis depends on the history of eating particular kinds of seafood, clinical symptoms and time of recovery. Lacking of good serologic and clinical tests for the diagnosis of CFP, could result in misdiagnosis of CFP as other forms of food poisoning [9]. Detection of toxin in remnants of food can be done only in special laboratories. It is usually not possible because of the unavailability of food for examination by the time that patient develops symptoms. It is thus very likely that past cases of CFP in Thailand have not been identified.

The commercial available test kit for diagnosis of CTX in fish meat, *Cigua–Check*, is a simple and reliable method and needs only a rice-gain-sized piece of blood-free fish flesh. Raw or cooked samples can be used for testing as the toxin is heat resistant. The

Table 2. Cigua-Check assay of fishes and fish fillets from three markets in Bangkok

Species (common name)	Total no. of fish	No. of responses (%)		
		Negative	Borderline	Positive
Scomberomorus commerson (Spanish mackerel)	16	16	0	0
Epinephelus sp. (grouper)	13	13	0	0
Lates calcarifer (barramundi)	10	10	0	0
Epinephelus fasciatus (red-banded grouper)	4	4	0	0
Trichiurus lepturus (largehead hairtail)	3	3	0	0
Sphyraena barracuda (great barracuda)	3	3	0	0
Lutjanus malabaricus (Malabar red snapper)	3	3	0	0
Selaroides leptolepis (yellow stripe trevally)	2	2	0	0
Leiognathus fasciatus (common ponyfish)	2	2	0	0
Eleutheronema tetradactylum (fourfinger threadfin)	2	2	0	0
Lethrinus ornatus (ornate emperor)	2	2	0	0
Lactarius lactarius (false trevally)	1	1	0	0
Cromileptes altivelis (humpback grouper)	1	1	0	0
Arothron immaculatus (immaculate puffer)	1	1	0	0
Rastrelliger kanagurta (Indian mackerel)	1	1	0	0
Cynoglossus macrolepidotus (large-scale tonguesole)	1	1	0	0
Priacanthus tayenus (purple-spotted bigeye)	1	1	0	0
Rhynchobatus sp. (guitarfish)	1	1	0	0
Scomberoides lysan Forskal (yellow queenfish)	1	1	0	0
Pampus chinensis (Chinese pomfret)	1	1	0	0
Pomadasys hasta (lined silver grunt) bag No. 1 (03/07/2009)*	26 pieces	26	0	0
Pomadasys hasta (lined silver grunt) bag No. 2 (03/15/2009)*	26 pieces	26	0	0
Pomadasys hasta (lined silver grunt) bag No. 3 (06/15/2009)*	25 pieces	24	0	1 (4.0)
Pomadasys hasta (lined silver grunt) bag No. 4 (06/21/2009)*	27 pieces	26	0	1 (3.7)
Pomadasys hasta (lined silver grunt) bag No. 5 (06/21/2009)*	27 pieces	27	0	0
Pomadasys hasta (lined silver grunt) bag No. 6 (07/12/2009)*	27 pieces	27	0	0
Total	227	225 (99.1)	0 (0)	2 (0.9)

*Date of sample collection

test can be completed within 50 minutes. This kit is a membrane immunobead assay (MIA). It is based on an immunological principal, using monoclonal antibody to purify CTX and colored polystyrene beads and hydrophobic membrane laminated onto a solid plastic support [21]. The MIA was firstly developed in 1988, it was evaluated in 13 fish implicated in human CFP, 12 (92.3%) of them showed borderline (2 specimens) or positive (10 specimens) responses in the assay. The one MIA negative result showed weak CTX-like toxin in a guinea pig atrial assay; suggesting that its major toxic component to be other than CTX [21]. Hokama et al., in 1998, conducted an examination of 154 routinely caught reef fish by MIA assay and found that 132 fish samples or 85.7% were negative, 14 samples or 9.1% were borderline and 8 samples or 5.2% were positive [21]. No false negative responses were recorded after these MIA negative fish were eaten. Thus, the sensitivity (92.3%) and specificity (85.7%) values are within acceptable ranges for such a biological test system [21].

The Cigua-Check assay was performed for screening of CTX in our study. All critical factors to obtain accurate and reproducible results were followed as stated in the kit instructions and a previous study [21]. For example, the membrane must not be touched to avoid false positive reactions, the membrane stick must be soaked in the methanol/fish sample suspension for at least 20 min for optimal results and to prevent false positive results, the stick must be completely dry before adding it to the latex immunobead suspension and it should not be soaked for more than 10 min.

CFP causes a potential threat not only to public health, but also to the local seafood related businesses and to tourism. In 1984, there was one reported case of ciguatoxin poisoning in a 29-year-old Italian woman who had ingested marine fish during her travel in Thailand [22]. She complained of gastrointestinal and neurological disturbances, marked by severe, mainly motor, demyelinating polyneuropathy, which worsened on her return to Italy a few days later. The clinical pattern, electromyography, cerebrospinal fluid test and sural nerve biopsy by electron microscopy resulted in the diagnosis of polyneuropathy secondary to ciguatoxin poisoning. In 1980, 20 types of fish from fish markets in Bangkok, Chantaburi, and Chonburi were collected for testing for CTX by mouse bioassay [23]. CTX was not detected from any of the meat and livers of these fish tested by the mouse bioassay method.

At least three fish species (grouper, barracuda, and snapper), that have been reported as a source of CTX in endemic areas were included in our study. Nineteen samples from these three species gave negative results on CTX testing by Cigua-Check assay. This may be attributed to the fact that the number of samples was too small, or it was not fish from CTX risk areas. Source of fish could not be identified in this study because it was randomly sampled from the market. Two CTX-positive fish samples were found in this study. Triplicate repeats of these samples were performed and all three tests showed the same level of positivity. These two samples were from the separate fillet pack of lined silver grunt. Their origin is not known. Finding positives only from the fish fillets, but not from the individual marine fish, may be caused from the bias of the higher number of fish in fillet samples. Fish fillet is very popular at Thai household and restaurants because there is no bone and it is ready to be cooked (**Figure 1**). However, the lined silver grunt was never reported as a source of CTX. These results thus require further surveillance with more precise confirmation tests such as a mouse bioassay, guinea pig atrial assay, or mass spectrometry methods. The origin of the samples also needs to be identified, which is a difficult to impossible task in a huge busy market.

Prevention of ciguatera intoxication is difficult because of the colorless, odorless and tastelessness of CTX. Most importantly, CTX is resistant to heat and cannot be destroyed by cooking. Current recommendations are that one should avoid eating fish or internal organs of high risk fish, such as moray eel, barracuda, grouper, kingfish, jacks, snapper, surgeonfish, parrot fish, wrasses, hogfish, narrow barred Spanish mackerel, coral trout, flowery cod, and red emperor [24]. If known fish are not avoidable, it has been suggested to consume only small amounts of unknown types of fish (<50g) and it has also been suggested to select only small fish (less than 2 kg body weight), which may reduce the risk of intoxication.

Surveillance of CTX in fish markets can provide preliminary information for control of CTX outbreaks because it provides preconsumption indication of CTX findings. This type of surveillance is conducted in many studies of countries on a research basis. It can be used as monitoring measure for CTX surveillance.

Figure 1. Fish fillets from lined silver grunt

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