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Emotional and motor symptoms in riverside dwellers exposed to mercury in the Amazon

Manifestações emocionais e motoras de ribeirinhos expostos ao mercúrio na Amazônia

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ABSTRACT: ***Introduction:*** The investigation of clinical and neurological impactations associated with exposure to mercury levels in exposed populations is necessary in the Amazon. ***Objective:*** To analyze emotional and motor symptoms of riverside dwellers exposed by diet in the municipalities of Itaituba and Acará, in Pará, Brazil. ***Methods:*** Hair samples were collected to assess the total mercury (HgT). Demographic data as well as emotional (depression, anxiety and insomnia) and motor (paresthesia, muscle weakness, loss of balance when walking, tremors, limb pain and dysarthria) symptomatology data were obtained. ***Results:*** Mean levels of HgT in Itaituba were significantly higher ($p < 0.0001$) than in Acará. Emotional symptoms were identified in 26 (26.5%) participants from Itaituba and in 24 (52.2%) from Acará. Specific motor complaints in Itaituba occurred in 63 (64.3%) volunteers; the most frequently mentioned afflictions were limb pain (36.7%), paresthesia (32.6%) and muscle weakness (27.5%). In Acará, 33 (71.7%) participants had motor symptoms, the majority of which complained of paresthesia (54.3%), limb pain (52.2%) and tremors (34.8%). Average HgT levels in Itaituba in those with emotional and motor symptoms were above the tolerable levels (6 μg/g) determined by the World Health Organization. ***Conclusion:*** Results showed that mercury levels in emotional and motor symptoms in Itaituba are higher than in riverside dwellers in Acará. Further studies, including the application of specific qualitative and/or quantitative standard tests, as well as the investigation of other clinical signs are necessary.

Keywords: Mercury. Amazon ecosystem. Environmental exposure. Toxicity. Mercury poisoning.

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RESUMO: ***Introdução:*** A investigação dos impactos clínico-neurológicos associados às concentrações de exposição ao mercúrio em populações expostas é necessária na Amazônia. ***Objetivo:*** Analisar as manifestações emocionais e motoras de ribeirinhos expostos pela dieta nos municípios de Itaituba e Acará, ambos no Pará. ***Método:*** Foram coletadas amostras de cabelo para a determinação de mercúrio total (HgT), obtidos dados demográficos e sintomatológicos emocionais (depressão, ansiedade e insônia) e motores (parestesia, fraqueza muscular, desequilíbrio ao andar, tremor, dor nos membros e disartria). ***Resultados:*** A concentração mediana de HgT em Itaituba foi significativamente superior ($p < 0,0001$) àquela em Acará. As manifestações emocionais foram identificadas em 26 (26,5%) participantes de Itaituba e em 24 (52,2%) em Acará. Com relação às queixas motoras especificas, em Itaituba ocorreram em 63 (64,3%) voluntários, sendo mais referidas a dor nos membros (36,7%), a parestesia (32,6%) e a fraqueza muscular (27,5%). No Acará, 33 (71,7%) participantes apresentaram manifestações motoras, com o maior número queixando de parestesia (54,3%), dor nos membros (52,2%) e tremor (34,8%). As concentrações médias de HgT em Itaituba naqueles com manifestações emocionais e com manifestações motoras estiveram acima do considerado tolerável (6 μg/g) pela Organização Mundial de Saúde. ***Conclusão:*** Os resultados revelaram que a concentração de mercúrio nas manifestações emocionais e motoras de Itaituba são maiores do que nos ribeirinhos do Acará. Novos estudos são necessários com a aplicação de testes convencionais qualitativos e/ou quantitativos específicos, assim como também a investigação de outros sinais clínicos.

Palavras-chave: Mercúrio. Ecossistema Amazônico. Exposição ambiental. Toxicidade. Intoxicação por mercúrio.

INTRODUCTION

The incident in Minamata Bay, in Japan, in the 1960s, which had major international repercussions, establishes the toxic potential of mercury as a fish contaminant. The dumping of by-products from chemical factory directly into the bay[1] caused environmental contamination. The case provided an important body of information on the clinical symptoms and epidemiological characteristics of mercury poisoning, serving as a milestone for the mobilization of organized segments of civil society in different parts of the world, including Brazil[2].

In the Amazon region, several studies monitor the levels of mercury exposure in the population[3-11] and the fish [12-14], as the region presents a consequent contamination resulting from mineral prospecting, forest fires, the presence of large natural reservoirs and the implantation of hydroelectric plants that cause flooding, transferring mercury from the soil to the water column[15].

Mercury (Hg) as a fish contaminant is a form of non-occupational exposure; its concentration occurs in the organic form of methylmercury (MeHg). The toxicity of this compound especially affects the nervous system, causing muscular and nervous dysfunctions that may lead to irreversible damages or death, as stated by Carta et al.[16]. Therefore, the World Health Organization (WHO) determines that Hg levels above 50 μg/g in a person's hair is a risk to the adult nervous system[17].

Studies have shown that acute exposure to MeHg may cause weakness, fatigue, weight loss, emotional instability, mental confusion, psychiatric disorders, concentration problems, amnesia, tachycardia, delirium, dizziness, motor incoordination, paresthesia, ataxia, deafness, muscle tremors, visual field constriction, coma and even death[18,19]. Chronic toxicity is characterized by sensory loss in the extremities, dysarthria, muscle weakness, irritability, decreased attention and memory, anxiety, depression, insomnia, coordination and balance disorders, all motor signs that simulate amyotrophic lateral sclerosis and seem to increase the risk for cardiovascular diseases[20-22].

In the Amazon region, the riverside-dwelling communities that are exposed to Hg through ingestion of contaminated food are unaware of any negative health impacts that this exposure may cause. There is evidence that communities which regularly have fish as part of the diet display levels of Hg exposure that pose health hazards[23]. With that, investigations into clinical symptoms associated with exposure levels have been the subject of research in exposed populations[4,6,24]. The Hg poisoning diagnosis should be based not only on the epidemiological findings and the high Hg levels in blood or hair samples, but also on clinical findings as stated by Harada et al.[25] and Pinheiro et al.[26].

This study aimed at analyzing emotional and motor symptoms of individuals exposed to MeHg through their diet, contributing to the beginning of the epidemiological, toxicological and clinical monitoring of this special group.

METHODS

STUDY LOCATION

The study included riverside dwellers from communities known as Barreiras and São Luiz dos Tapajós, in the municipality of Itaituba, Pará, and Furo do Maracujá, in the municipality of Acará, Pará.

Located in the southwest region of the state of the Pará, the municipality of Itaituba is on the left bank of the Tapajós River, bordering the state of Amazonas. Its geographical coordinates are 04º 16' 34''S and 55º 59' 06''W. Its estimated population, according to the Brazilian Institute of Geography and Statistics (IBGE)[27], has 98,493 inhabitants, including the population that is part of the city and the communities distributed on the banks of the river. Historically, the municipality is influenced by gold prospecting.

The municipality of Acará is part of the micro region composed by the municipalities of Concórdia do Pará, Tomé Açu, Bujaru, Tailândia and Moju. It is at 01º 57' 39"S and 48º 11' 48"W. It is composed by a total population estimated at 53,569 inhabitants, who use local fish as the main source of protein in their diet and part of survival through the basic extraction of the acai crop, without any known mineral prospecting[27].

STUDY POPULATION

Men and women between the ages of 13 and 53 who have permanently resided in the community for more than 1 year participated in the study. Individuals who had acute musculoskeletal disorders in the upper and/or lower limbs with previously diagnosed neurological diseases and those who had recent exposure to Hg vapor were excluded from it.

TOTAL MERCURY DETERMINATION

To determine total mercury (HgT), approximately 10 to 20 mg of hair were collected from each volunteer, which were then placed in a paper envelope with identification and sent to the Human & Environmental Toxicology Laboratory of the Center for Tropical Medicine (NMT) of the Federal University of the Pará (UFPA)[28] for analysis.

In the laboratory, the hair samples were rinsed with neutral detergent, washed intensely with deionized water to remove the detergent and finally washed with 3 mL of acetone and dried at room temperature. Afterwards, the samples were cut so powder micro fragments were obtained[28]. About 10 mg were used between layers of calcium carbonate, calcium hydroxide and aluminum hydroxide to be analyzed by atomic absorption spectrophotometry with gold foil amalgamation using an automatic mercury meter called Mercury Analyzer (MA), SP-3D model by Nippon Corporation Japan[28].

The accuracy of the HgT analyses was determined by means of the duplicate quantification and the accuracy established by International Reference Standard IAEA 085. Reproducibility was demonstrated by linearity $r = 1$ through a calibration curve consisting of 5 points (0, 10, 20, 50 and 100). Results were expressed as $\mu g/g$[28].

All these steps followed the recommendations of the analytical instrument manufacturer (NIC Corporation)[28] and are also used by Milhomem Filho[29], Corvelo et al.[9], Pinheiro et al.[23], Costa Junior et al.[10] and Lima et al.[11].

DEMOGRAPHIC DATA AND SUBJECTIVE SYMPTOMATOLOGY

The clinical and epidemiological data, recorded in a specific form, were obtained during anamnesis and the neurological clinical examination performed by a neurology specialist, in a reserved space, at the community health care center, after receiving the participant's informed consent form. Demographic variables included sex, age, length of residence and occupation of participants. Regarding clinical variables, emotional symptoms such as depression, anxiety, insomnia and motor symptoms such as paresthesia, muscle weakness, loss of balance when walking, tremors, limb pain and dysarthria were investigated. The meaning of each symptom was previously explained so volunteers, upon being questioned, could answer if they had the investigated symptom or not correctly.

STATISTICAL ANALYSIS

The information was catalogued in a protocol form to enable the creation of charts and graphs. Using the Bio Estat 5.0 statistical packet[30], the data were analyzed adopting $\alpha = 0.05$ or 5% with a 95% confidence interval (95% CI) for statistical significance analysis. The results of the HgT levels, being continuous quantitative variables, were expressed by measures of central tendency (arithmetic mean and median) and dispersal (standard deviation). Demographic data and symptomatology were adjusted by frequency and percentage because variables were classified as qualitative nominal variables. The Mann-Whitney U test, a non-parametric test, was used to compare the median HgT levels between municipalities. To analyze the measure of association as an odds ratio of the emotional and motor symptomatological findings between municipalities, the odds ratio (OR) was used.

ETHICAL ASPECTS

The study was developed according to Resolution No. 466/2012 of the National Health Council (CNS), Ministry of Health, Brazil, and approved by the Research Ethics Committee (CEP) of the NMT of the Federal University of Pará through Protocol No. 002/2011.

RESULTS

A total of 144 riverside dwellers participated in the study, of which 98 were volunteers from Itaituba and 46 from Acará.

In Itaituba, most riverside dwellers were female (76.5%). As for age, the highest frequency (34 riverside dwellers) was observed in the 24 to 35 year-old group (34.7%). Sixty-one (62.3%) riverside dwellers had been living in the area for at least 15 years; most of these participants were homemakers (42.9%). In Acará, males were less present (39.1%); 30 (65.2%) represented an age group older than 24 years; 17 (36.9%) had a length of residence of 15 to 28 years; and of the 46 riverside dwellers, 17 (37.0%) were homemakers, followed by 15 (32.6%) farmers and only one (2.2%) fisherman (Table 1).

The average HgT concentrations in the municipalities is shown in Table 2. HgT levels in Itaituba varied from 0 to 41.8 μg/g, with an average of 9.15 ± 8.17 μg/g. In Acará, the variation ranged from 0 to 2.34 μg/g in hair samples, with an average of 0.67 ± 0.54 μg/g. When comparing HgT levels in riverside-dwelling communities, there was a highly significant difference ($p < 0.0001$) among the assessed municipalities (Table 2).

Motor complaints were more frequent than emotional complaints in both municipalities; in Table 3, there is the frequency of emotional symptoms in each location. Emotional symptoms were identified in 26 (26.5%) riverside dwellers from Itaituba and in 24 (52.2%)

Table 1. Distribution of the population by sex, age group, length of residence and occupation, according to each location.

Demographic data	Itaituba [n = 98 (%)]	Acará [n = 46 (%)]
Sex		
Male	23 (23.5)	18 (39.1)
Female	75 (76.5)	28 (60.9)
Age group (years)		
13 ⊢-- 24	22 (22.4)	16 (34.8)
24 ⊢-- 35	34 (34.7)	13 (28.3)
35 ⊢-- 46	23 (23.5)	10 (21.7)
46 ⊢-- 57	19 (19.4)	7 (15.2)
Length of residence (years)		
02 ⊢-- 15	37 (37.7)	13 (28.3)
15 ⊢-- 28	23 (23.5)	17 (36.9)
28 ⊢-- 41	25 (25.5)	11 (23.9)
41 ⊢-- 54	13 (13.3)	5 (10.9)
Occupation		
Homemaker	42 (42.9)	17 (37.0)
Fisherman	11 (11.2)	1 (2.2)
Farmer	7 (7.1)	15 (32.6)
Student	16 (16.3)	7 (15.2)
Other	22 (22.5)	6 (13.0)

Table 2. Total mercury levels in hair (μg/g) presented by the riverside-dwelling communities in each municipality.

Variable	Itaituba [n = 98]	Acará [n = 46]	p-value*
Total mercury (μg/g)			
Average ± SD	9.15 ± 8.17	0.66 ± 0.54	< 0.0001
Median	7.10	0.60	
25th percentile	3.72	0.20	
75th percentile	11.25	0.90	
Minimum	0.00	0.00	
Maximum	41.80	2.34	

SD: standard deviation; *Mann-Whitney.

from Acará. The group with only insomnia (nine riverside dwellers) was the most representative (34.6 %) in Itaituba; there was only one case of insomnia and depression (3.9 %). In Acará, the largest representativeness (37.5 %) was observed in the group with only anxiety (nine riverside dwellers); no riverside dwellers were found only with depression. Two riverside dwellers (7.7 %) from Itaituba complained of the three symptoms, while in Acará, this fact was not found. In the comparison of groups, there was a significant difference between those only with anxiety and those with insomnia and anxiety ($p < 0.05$), with higher odds in Acará. Among those only with insomnia (OR = 1.06; 95% CI 0.31 – 3.64), there was a strong association with similar odds of symptoms in riverside dwellers from Itaituba and Acará ($p = 0.83$).

In Itaituba, specific motor complaints (Table 4) occurred in 63 (64.3%) riverside dwellers; the most mentioned were limb pain, paresthesia and muscle weakness. Of the 32 (32.6%) cases observed with paresthesia, 15 (15.3%) had upper- and lower-limb pain; muscle weakness was more connected to the lower limbs. In Acará, paresthesia, limb pain and tremors were the most frequent, in 33 (71.7 %) riverside dwellers. Loss of balance when walking, in both municipalities, was the less frequent complaint, and the presence of dysarthria was not observed in any riverside dwellers. Comparing the two municipalities with regard to subjective complaints, there was statistical significance for paresthesia, loss of balance when walking and tremors ($p < 0.05$), with a higher probability in Acará.

When comparing the median HgT levels in the municipalities of participants with complaints (Table 5), there were significant differences ($p < 0.0001$) in both emotional and motor aspects; the higher average Hg levels occurred specially in Itaituba, mainly in participants with motor complaints.

Table 3. Frequency of emotional symptoms according to each location.

Symptom	Itaituba [n = 26(%)]	Acará [n = 24(%)]	OR (95% CI)	p-value
Only insomnia	9 (34.6)	4 (16.7)	1.06 (0.31 – 3.64)	0.83
Only depression	2 (7.7)	0 (0.0)	–	–
Only anxiety	5 (19.2)	9 (37.5)	0.22 (0.07 – 0.70)	0.01
Insomnia + depression	1 (3.9)	1 (4.2)	–	–
Insomnia + anxiety	5 (19.2)	8 (33.3)	0.25 (0.08 – 0.83)	0.04
Depression + anxiety	2 (7.7)	2 (8.3)	0.46 (0.06 – 3.36)	0.81
Insomnia + depression + anxiety	2 (7.7)	0 (0.0)	–	–

OR: odds ratio; 95% CI: 95% confidence interval.

Table 4. Frequency of motor symptoms according to each location.

Symptom	Itaituba [n = 98(%)]	Acará [n = 46(%)]	OR (95% CI)	p-value
Paresthesia	32 (32.6)	25 (54.3)	0.41 (0.20 – 0.83)	0.02
UL paresthesia	13 (13.3)	10 (21.7)	–	–
LL paresthesia	4 (4.1)	5 (10.9)	–	–
UL + LL paresthesia	15 (15.3)	10 (21.7)	–	–
Muscle weakness	27 (27.5)	15 (32.6)	0.78 (0.37 – 1.68)	0.67
UL weakness	9 (9.2)	3 (6.5)	–	–
LL weakness	10 (10.2)	5 (10.9)	–	–
UL + LL weaknesses	8 (8.2)	7 (15.2)	–	–
Loss of balance when walking	3 (3.1)	6 (13.0)	0.21 (0.05 – 0.88)	0.05
Tremors	17 (17.3)	16 (34.8)	0.39 (0.18 – 0.88)	0.03
Limb pain	36 (36.7)	24 (52.2)	0.53 (0.26 – 1.08)	0.12
Dysarthria	0 (0.0)	0 (0.0)	–	–

OR: odds ratio; 95% CI: 95% confidence interval; UL: upper limbs; LL: lower limbs.

Table 5. Mercury levels (µg/g) of those with emotional and motor symptoms in each location.

	Itaituba [n = 98]	Acará [n = 46]	p-value*
Total mercury (µg/g) – emotional symptom			
Average ± SD	7.38 ± 4.70	0.66 ± 0.57	< 0.0001
Median	6.65	0.60	
25th percentile	4.07	0.18	
75th percentile	9.15	0.89	
Minimum	0.90	0.01	
Maximum	23.60	2.34	
Total mercury (µg/g) – motor symptom			
Average ± SD	8.84 ± 7.11	0.64 ± 0.58	< 0.0001
Median	7.40	0.59	
25th percentile	3.65	0.13	
75th percentile	11.61	0.78	
Minimum	0.02	0.00	
Maximum	32.50	2.34	

SD: standard deviation; *Mann-Whitney.

DISCUSSION

One of the routes of exposure to Hg in the Amazon region is the ingestion of contaminated food, more precisely the fish riverside-dwelling and fishing communities use as food menu option. The Hg levels in hair samples is considered the best bioindicator of exposure to Hg from ingestion of contaminated food [17] and is commonly used by several authors[5,7-11,23,29] to measure long-term exposure.

Through the different hydrographic locations it is possible to identify Hg exposure variations in each region. In these studies, the Hg levels in Itaituba were significantly higher ($p < 0.0001$) than those in Acará. The riverside-dwelling communities in the Tapajós region have a history of long-term exposure, for approximately two decades, and are known for uncontrolled mineral exploration[5-8,26]. Riverside dwellers in Acará had low HgT levels, which can be explained by the location, which is far from mineral prospecting, and by fish consumption with low HgT levels.

There was a prevalence of female participants in relation to men in both municipalities as well as a larger number of riverside dwellers in a reproductive age group, suggesting that, in the Tapajós region, exposures to HgT present a risk due to the relationship between mother and fetus. Based on the maternal and infant risk associated to exposure to Hg, a maximum safety limit of 10 μg/g in hair samples[17] is set.

In Itaituba, mercury levels ranged from 0 to 41.8 μg/g with an average of 9.15 ± 8.17 μg/g, which is above the established safety limit for regular fish consumption[31]. Recent studies have revealed a similar reality. Vieira et al.[32], in the riverside-dwelling communities of the Madeira River, Amazonas, found average concentrations of 8.02 μg/g. Lima et al.[11] identified average concentrations of 7.98 μg/g in riverside dwellers living in villages in the Tapajós. Costa Junior et al.[10], assessing the exposure evolution in riverside-dwelling communities in the Tapajós region, found average concentrations below 10 μg/g in 2012 and 2013. Pinheiro et al.[23], assessing the frequency of neurological signs and symptoms in the riverside-dwelling population in the Tapajós River Basin, observed variations of mercury levels ranging from 0.87 to 44.59 μg/g.

Concentration averages higher than those found in this study have already been reported by other authors[3-6,26,33], but in periods when mineral prospecting was intense and the preventive educational activities were gaining strength. High Hg levels may be connected to the population's amount and type of fish consumption and to the lack of variation in their diet. Passos et al.[33], when assessing the effects of fruit consumption on the relationship between fish consumption and mercury bioindicators, concluded that fruit consumption may provide a protective effect for exposure to Hg in riverside dwellers in the Amazon region.

Insomnia, depression and anxiety are characteristic signs and symptoms of Hg intoxication and are reported by Chuu[21] and Chang[22]. Emotional symptoms were identified in 26 (26.5%) riverside dwellers from Itaituba and in 24 (52.2%) from Acará. Comparing locations,

the statistical difference ($p < 0.05$) was found among those only with anxiety and among those with anxiety and insomnia. The frequency of individuals with such results was higher in Acará, suggesting that the exposure to Hg is not the causal factor, since Hg levels were not elevated in this municipality.

Psychic changes typical of erethism mercurialis, such as insomnia, depression and anxiety, were present in communities exposed to Hg. However, other clinical signs such as irritability, melancholy, shyness, indecision, concentration problems, intellectual dulling and changes of character and personality should be investigated to justify these clinical syndromes.

Regarding motor complaints, neurological symptoms similar to those in the Minamata disease, in Japan, were found in this study. In Itaituba, an area exposed to Hg, the three most frequent complaints were paresthesia, limb pain and muscle weakness. Carta et al.[16] also they described the same symptoms in riverside dwellers in the Tapajós River Basin, which had HgT levels above 20 μg/g in the hair. Muscle fatigue was also identified by Lebel[3] when investigating exposed populations in the Amazon, concluding that there is a positive correlation between exposure to methylmercury and neurological abnormalities.

In this study, the tremors found in 17 riverside dwellers in the Tapajós region was found by Auger[24] in adult Cree aborigines in northern Quebec, especially young adults; these tremors are associated with high methylmercury levels, but below 50 μg/g. Dysarthria was not noticed in any of the group; however, it was present in the results of Harada et al.[6] when observing fishing villages in the Tapajós River Basin, in the Itaituba municipality.

Two major environmental disasters recorded numerous cases of methylmercury poisoning, which took place in Iraq and in the city of Minamata, in Japan. In both tragedies, cases of muscle weakness, paresthesia, generalized pain and tremors were recorded[34]. In Iraq and Japan, exposure was predominantly acute, resulting from high methylmercury levels. In this study, motor complaints in Itaituba occur in communities where exposure has been occurring in the long term and Hg levels are below the risk value for the adult nervous system established by WHO[17]. Therefore, the low frequency of neuromuscular symptoms among riverside dwellers in the Tapajós may be explained by exposure intensity, which was still not enough to cause motor damage. The highest frequency of motor complaints in Acará suggests that there is no relation to Hg, and these may be related to other factors not assessed in this study.

The average concentrations of Hg were higher in Itaituba, especially in participants with motor complaints. A similar fact was found by Dolbec[4] when studying the effects of methylmercury on the motor functions of Amazonian populations, observing the association of neurobehavioral symptoms with exposure. Lebel[3], when evaluating the effects of methylmercury on the health of a riverside-dweller population in a village in the Tapajós River, noticed that there was a tendency of motor implications according to the increase of Hg levels in the hair.

The results of this study contribute to the identification of possible clinical symptoms related to exposure, since little is known about the impacts and effects of long-term exposure to low doses of methylmercury in adults in exposed riverside-dweller populations, as stated by Auger[24]. They also provide new relevant information about the emotional and physical condition of the participating population, since clinical findings are important for the poisoning diagnosis.

CONCLUSION

Results showed that Hg levels in emotional and motor symptoms of riverside dwellers in Itaituba, an area with indications of contamination, especially by gold mining, is higher than in riverside dwellers in Acará. The identification of clinical findings with a higher frequency of motor complaints than emotional complaints in Itaituba reiterate methylmercury toxicity in relation to the central nervous system. However, further follow-up studies are necessary, making the monitoring of concentrations critical and including the investigation of other clinical signs, since the exposure to Hg can affect several other systems.

Assessments using subjective methods are positive tools for the initial identification of symptoms due to Hg poisoning and may be an alternative form of clinical follow-up. Nevertheless, objective quantitative or qualitative methods are necessary and essential for the confirmation of clinical findings.

The search for Hg levels, epidemiological aspects and the clinical condition is crucial to strengthen preventive, assessing and educational measures in areas with an environmental pollution impact so that consequences such as those seen in the Minamata tragedy in Japan do not affect the health of the Amazon population.

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