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Folic Acid Intake and Neural Tube Defects: Two Egyptian Centers Experience

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Abstract: Neural tube defects (NTDs) are a group of congenital malformations with worldwide distribution and complex etiopathogenesis. Folic acid plays a pivotal role in their prevention. We aimed to identify the protective effect of folic acid intake against NTDs and its dependence on different socioeconomic and environmental factors in a cohort of mothers in Egypt.

A cross-sectional study was carried over a period of 12 months on mothers who gave birth to babies with NTDs (group 1) and a control group with healthy offsprings (group 2). Both groups completed 2 questionnaires: food frequency questionnaire targeting the daily folate intake, and socioeconomic status and medical history questionnaire.

Both groups of mothers received folate <800 µg/day, recommended for pregnant women. A strong association was detected between NTDs and urban residency with medium educated mothers, with negative consanguinity, who had folate intake < 400 µg daily, and who had their food long cooked. Each of these factors separately had a limited impact to cause NTDs, but when present together they did augment each other. Interestingly enough is the role of fava bean, cauliflower, spinach, and mango in predisposing of NTDs in the presence of the above-mentioned factors.

The protective effect of folic acid intake against NTDs may depend on the synergism of different socioeconomic and environmental factors (which differ from country to another). In Egypt, females especially the

medium-educated who live in urban areas should be well-informed with the value of folate intake in the periconceptual period.

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Abbreviations: NTDs = neural tube defects, RDA = recommended daily allowance, USDA = U.S. Department of Agriculture, FDA = Food and Drug Administration.

INTRODUCTION

It has been estimated that neural tube defects (NTDs) affect > 300,000 pregnancies per annum worldwide being an important cause of perinatal mortality and infantile paralysis.^{1,2} Severe NTD (a meningocele) has been described in a 4000-year-old Egyptian infant mummy; however, the role of folic acid insufficiency as a risk factor for NTD was only recognized in the 20th century.³ Approximately 4500 of these cases occur in Europe, at a rate of ~11 per 10,000 births in the UK and Ireland,⁴ with a similar rate in New Zealand.⁵ Although no precise figures on NTDs are available in Egypt, rough estimates of 4.26/1000 live born babies, and a relatively high incidence at Cairo University Children Hospital have been observed (unpublished data).

NTDs are unique in that the majority of cases are preventable by the adequate intake of folic acid before and during the first trimester of pregnancy.^{6,7} Internationally, this has led many governments to adopt guidelines recommending women to take ≥400 micrograms (µg) of folic acid daily during the periconceptual period and during pregnancy.⁸

In developed nations, almost half of all pregnancies are unplanned,⁹ representing a large proportion of pregnancies not amenable to effective supplementation.¹⁰ Furthermore, the uptake of folic acid supplementation is not universal among women who plan their pregnancies. The worldwide socio-demographic predictors of poor intake include young maternal age, minority or non-European ethnicity, and low education and income.^{11–14}

The life of children with NTDs is very difficult worldwide with a significant high burden of disease and associated costs on families and societies, which become much more magnified in a country like Egypt with limited public social support structures.

In developing countries like Egypt, it would be much logic and effective to apply the role of “prevention better than control” where the urge to estimate the problem of NTDs and its underlying factors including adequacy of folate intake and in turn settle local preventive programs. Accordingly, this research was designed on a cohort of Egyptian mothers to figure out the possible contributing sociodemographic and environmental factors leading to NTDs, and the preventive roles of periconceptual folate intake together with promoting attitudes toward healthy lifestyle. The result of such a research might help implement better preventive strategies.

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PATIENTS AND METHODS

A case-control study was conducted in the outpatient and inpatient services of Cairo University Children Hospital, the biggest tertiary care pediatric hospital in Cairo, Egypt, and Benha University Hospital over a period of 12 months, January 2013 to January 2014, on a cohort of mothers who gave birth to babies with NTDs (group 1) and a control group of mothers who delivered healthy babies (group 2). We included only mothers whose infants ≤6 months old who agreed to participate in the study. Mothers of infants with NTDs associated other major congenital anomalies or syndromes were excluded.

Considering the 1 year time frame of the study and the availability of the cases in the 2 hospitals, the sample size was convenient.

All mothers were interviewed using 2 types of questionnaires as a retrospective survey. (1) Food Frequency Questionnaire (FFQ) for evaluation of their daily intake of folate from food/week assessing how often mothers ate different types of commonly available food during the periconceptional period of the corresponding pregnancy, and (2) socio-demographic and medical questionnaire along with questions about inter-family relationship and medicinal folate supplementation.

With the difficulty to recall the exact amount of the food taken, we classified the participants as: +ve meaning frequent consumers of commonly available foods and -ve meaning infrequent consumers of such foods.

The folic acid content of the frequently consumed common foods is estimated using the USDA National Nutrient Database for Standard References.¹⁵

The study was approved by both hospitals review boards. A written informed consent was signed by the contributing women with full explanation of the study protocol and for the use of their data for future research.

Statistical Analysis

Data analysis was based on SPSS version 10.0 for Windows with Excel and Nutri-survey 2007. Parametric data were statistically described in terms of range and mean ± standard deviation (SD). Nonparametric data were described as

frequencies (number of cases) and percentages when appropriate. The comparison of parametric variables between the studied groups was done using unpaired Student *t* test. For nonparametric data, the comparison was done using the chi square (X^2) test, Odd and risk ratios. Multivariate analyses were done using 2 steps first correlation matrix followed by regression analysis. The probability test was considered significant when *P* values were < 0.05.

RESULTS

The present study included 62 women with babies having NTDs and 118 control women with healthy offspring. Their age ranged between 16 and 43 with a mean of 25.7 years and 17 and 35 with a mean of 25.8 years, respectively. Their demographic data are listed in Table 1, where significantly higher weight was demonstrated among mothers of NTDs group. No significant differences were demonstrated regarding maternal age, infant's age, and daily folate intake which was in both groups less than the 800 µg recommended for pregnant females.

The social, family, conception, and medical histories are listed in Table 2 and Figure 1, which highlight a statistically significant higher prevalence of NTDs in urban, medium-education, long cooking of food, and maternal diabetes. But strange enough, there was statistically significant low association between existing family relationship and NTDs.

Analysis of different foods taken by mothers with NTDs is demonstrated in Table 3, which surprisingly reveals a significant association between intake of fava beans, cauliflower, spinach, and mango and occurrence of NTDs, whereas the consumption of oranges showed a protective role. Coriander had statistically insignificant low association between its intake and occurrence of NTDs only but statistically significant high odd ratio.

A multivariate regression for determining the most important determinants of NTDs is demonstrated in Table 4. Residency associated with low education, long cooking, maternal diabetes mellitus, overweight, and eating mango are the strongest determinants for NTDs. It highlights that NTD's occurrence is an environmentally multifactorial outcome resulting

TABLE 1. Demographic Data of the Studied Groups and Daily Folate Intake

Item	Mothers of NTDs patients	Control Mothers	<i>P</i>	Total
Mat. age (years)				
Mean ± SD	25.6 ± 7.6	25.8 ± 4.9	>0.05	25.7 ± 6.3
Median	22.3	24.5		24.3
95% CI	16–43	24.3–27.3		22–29
Infant's age (days)				
Mean ± SD	72.9 ± 32.1	79.9 ± 39.5	>0.05	76.4 ± 35.8
Median	53	58		55
95% CI	18–127	43.7–116.2		22–122
Weight (Kg)				
Mean ± SD	84.7 ± 13.4	61.2 ± 11.1	<0.0001	72.9 ± 12.3
Median	79	60		70
95% CI	48–97	47–75		47–96
Total daily folic acid food intake (µg)				
Mean ± SD	424.3 ± 155.4	411.4 ± 158.5	>0.05	417.8 ± 156.7
Median	392.8	359.9		376
95% CI	190.2–689.3	178–749		188–685

CI = confidence interval, NTDs = neural tube defects, SD = standard deviation.

TABLE 2. Social, Family, Conception, and Medical Histories of the Studied Population and Folate Intake

Item	Mothers of NTDs Patients		Control Mothers		Total	
	No.	%	No.	%	No.	%
Residency						
Urban	36	58.1	102	86.4	138	76.7
Rural	26	41.9	16	13.6	42	23.3
X ² = 4.8, P < 0.01 with lambda for predicting NTDs = 0.136 SE ± 0.34, 95% CI 0–0.8						
Education level						
Low	6	9.7	92	80	98	54.4
Moderate to high	56	90.3	26	20	82	45.6
X ² = 35.5, P < 0.0001 with lambda for predicting NTDs = 0.484 SE ± 0.28, 95% CI 0–1						
Family relations						
Absent	26	41.9	20	16.9	46	25.6
Present	36	58.1	98	83.1	134	74.4
X ² = 13.2, P < 0.0003 with lambda for predicting NTDs = 0.097 SE ± 0.34, 95% CI 0–0.7						
Folate medicinal supplementation intake at conception						
No	14	22.6	32	27.1	46	25.6
Yes	48	77.4	86	72.9	134	74.4
X ² = 0.4, P > 0.05						
Daily food intake of folic acid (µg)						
<400	34	54.8	50	42.4	84	46.7
>400	28	45.2	68	57.6	96	53.3
X ² = 1.4, P > 0.05						
Food preparation						
Long cooked	60	96.8	82	69.5	142	78.9
Med. cooked	2	3.2	2	1.7	4	2.2
Short cooked	0	0	34	28.8	34	18.9
X ² = 11.1, P < 0.004						
Diabetes mellitus						
Absent	8	12.9	106	89.8	114	63.3
Present	54	87.1	12	10.2	66	36.7
X ² = 48.5, P < 0.0001						
Cesarean section						
No	14	22.6	16	13.6	30	16.7
Yes	48	77.4	102	86.4	150	83.3
X ² = 0.6, P > 0.05						
No. of siblings						
<3	46	74.2	86	72.9	132	73.3
>3	16	25.8	32	27.1	48	26.7
X ² = 0.6, P > 0.05						
Parity						
Primipara	40	64.5	36	30.5	76	42.2
Multipara	22	35.5	82	69.5	104	57.8
X ² = 8.3, P < 0.004						

CI = confidence interval, NTDs = neural tube defects, SE = standard error.

from the interaction of many variables, which separately and by themselves could not individually be the direct cause of NTDs.

DISCUSSION

The present study reinforces the association between the occurrences of NTDs and a low intake of folic acid associated with socio-demographic-economic variables. Being a world-wide problem, many agencies worked on it. The United States (US) Department of Health and Human Services declared as 1 of the 2 major goals of the Healthy People 2010 initiative “to eliminate health disparities among different segments of the

population.”^{16–17} In addition, the Food and Drug Administration (FDA) of the United States first authorized folic acid fortification of all enriched cereal-grain products in United States in March 1996 with the primary purpose of decreasing the number of pregnancies affected by NTDs, and the policy became mandatory by January 1998.¹⁸

Lynch et al¹⁹ have recently highlighted the importance of examining social disparities based on absolute risk measures such as the excess risk difference. All indicated that there is some evidence of socioeconomic differences in diet and nutrition. Lower income, lower educational attainment, and non-Hispanic black ethnicity are associated with lower intake of

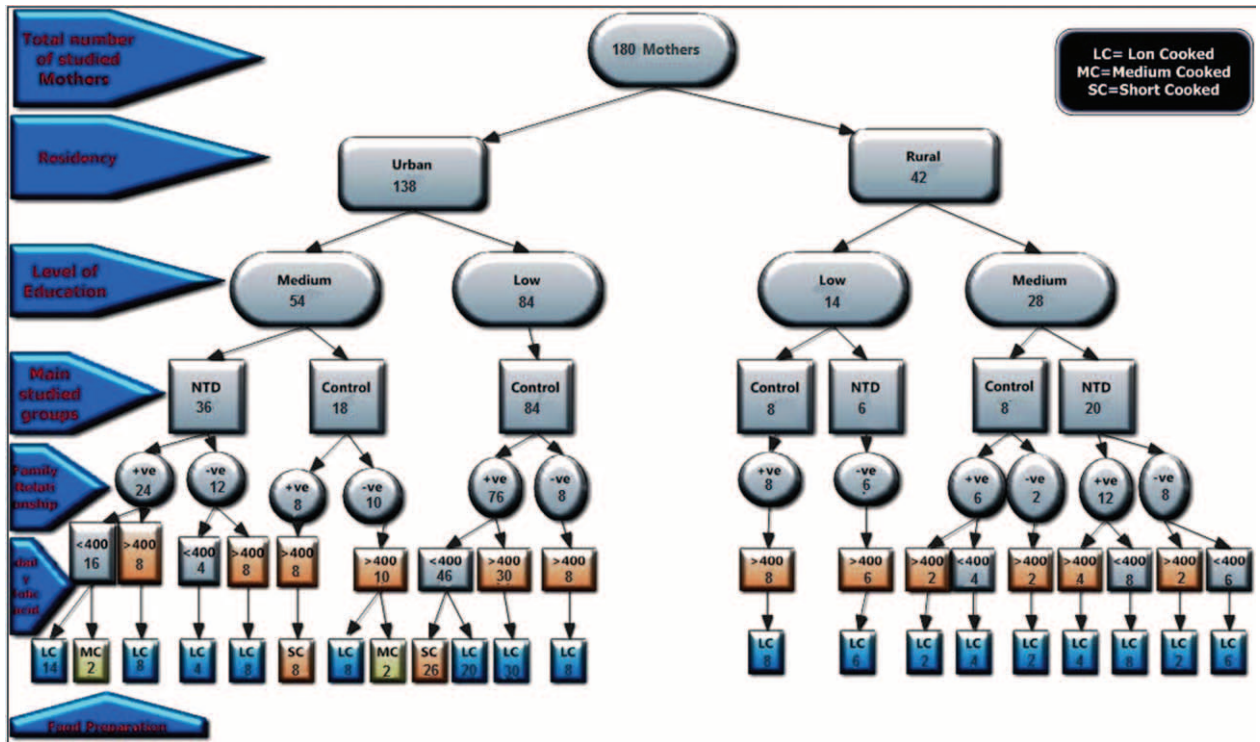


FIGURE 1. Flow chart of the study results.

fruits, vegetables and essential nutrients, and higher intake of total fat in the United States.^{20–21} The present study differs from the previous findings in the geographic distribution and education levels. This observation could be attributed to the fact that medium level educated mothers in urban Egypt are socially independent compared to those in rural areas with low education levels where families give help to young mothers.

In 2008, Stockley and Lund¹² reported unplanned pregnancy and younger maternal age as important factors associated with low folic acid intake. This finding is very similar to ours: mothers with younger age and primigravida were found to be associated with a higher—though not statistically significant—occurrence of NTDs. One explanation might be that pregnancy was often unplanned and probably periconceptional folic acid supplementation was inadequate.

An interesting finding in the present study is the fact that the well-known strong extended family relationship in Egyptian rural regions and in low socioeconomic areas exerted a protective role against NTDs, which were found significantly more frequent in the control group.

Table 2 highlights 3 important factors having a predictive value for NTDs in the Egyptian population where the lambda was found to be highest at the level of education, followed by geographic distribution, and then by family relationship. This means that folate deficiency resulted from interactions of combination of the different predisposing factors. If present alone neither of them would have an impact on folate intake and subsequently on NTD risk.

Studies examining a potential link between the period between pregnancies and occurrence of NTD have found mixed results.²² NTD's risk does not appear to be related to subfertility,²³ a finding that was not studied in the current investigation.

Recent work has shown that despite flour fortification, most women in this study have intakes of > 300 µg of folates but do not consume as much folic acid as to meet the Institute of Medicine's recommendation of 400 µg/day.^{16,24} As the European DACH-recommendations for the folate intake of adults have been lowered to 300 µg/day,²⁵ the Egyptian mothers in this study met this recommended daily allowance (RDA). Still, the study supports the recommendation of an additional intake of 400 µg folic acid in the periconceptional period for reducing the risk of NTDs.

Women with diabetes are at an increased risk for having an infant with a birth defect. The results of several studies suggest that this increased risk applies to NTDs in particular^{26–30} a finding that has been observed in the present study although the number of mothers with diabetes mellitus was too small for a conclusion. The present study revealed a statistically significant strong correlation between obesity and NTDs—a similar finding to that reported by other authors.^{31–32}

In the United States, many studies demonstrated that food folate is quite similar across racial/ethnic groups both before and after fortification whereas intake from supplements is dramatically higher in non-Hispanic whites.^{1,33–34} These findings are supported by the present study which found an insignificant difference between folate intake from food by the mothers with offspring affected by NTD and the control group. However, as shown in Figure 1, the distribution of mothers according to residency and education level could exaggerate the impact of daily folate intake in such a way that 55.6% of medium-level educated mothers living in urban areas showed daily folic acid intakes < 400 µg, and 70% in rural areas. On the other hand, 54.8% of low-educated urban mothers showed daily folic intake <400 µg. In the rural areas, the respective figure

TABLE 3. Prevalence of Certain Foods Containing Folic Acid in the Studied Groups

Item	Mothers of NTDs Patients		Control Mothers		P	Odd Ratio	Risk Ratio
	No.	%	No.	%			
Baladibread*							
-ve	8	12.9	4	3.4	>0.05	4.2	2.03
+ve	54	87.1	114	96.6			
Fava bean						0.13	0.7
-ve	2	3	24	20.3	<0.01	0.01–1.1	0.5–0.8
+ve	60	97	94	79.7		0.02	0.02
Taamiya†							
-ve	6	9.7	24	20.3	>0.05	0.4196	0.8
+ve	56	90.3	94	79.7		0.11–1.62	0.58–1.07
Lentils						0.5	0.8
-ve	14	22.6	42	35.6	>0.05	0.2–1.4	0.6–1.1
+ve	48	77.4	76	64.4		>0.05	>0.05
Egg						1.1	1.03
-ve	8	12.9	14	11.9	>0.05	0.3–4.1	0.6–1.7
+ve	54	87.1	104	88.1		>0.05	>0.05
Cheese							
-ve	0	0	6	5.9	>0.05		
+ve	62	100	112	94.1			
Yoghurt						1.9	1.2
-ve	48	77.4	76	64.4	>0.05	0.7–5.1	0.9–1.6
+ve	14	22.6	42	35.6		>0.05	>0.05
Milk						0.6	0.9
-ve	6	9.7	18	15.3	>0.05	0.15–2.4	0.59–1.2
+ve	56	90.3	100	84.7		>0.05	>0.05
Cabbage							
-ve	2	3.2	6	5.2	>0.05		
+ve	60	96.8	112	94.9			
Cauliflower						0.301	0.71
-ve	10	16.1	46	39	<0.05	0.10–0.9	0.54–0.93
+ve	52	83.9	72	61		<0.05	<0.05
Chickpea						0.531	0.8
-ve	26	41.9	68	57.6	>0.05	0.2–1.3	0.6–1.1
+ve	36	58.1	50	42.4		>0.05	>0.05
Cucumbers							
-ve	8	12.9	6	5.1	>0.05		
+ve	54	87.1	112	94.9			
Green Mallow							
-ve	36	58.1	86	72.9	>0.05	0.51	0.78
+ve	26	41.9	32	27.1		0.21–1.3	0.54–1.13
						>0.05	>0.05
Jews Mallow							
-ve	0	0	8	6.8	>0.05		
+ve	62	100	110	93.2			
Okra						0.7	0.9
-ve	10	16.1	26	22	>0.05	0.21–2.12	0.6–1.2
+ve	52	83.9	92	78		>0.05	>0.05
Paprika						1.6	1.2
-ve	14	22.6	18	15.3	>0.05	0.5–4.9	0.8–1.9
+ve	48	77.4	100	84.7		>0.05	>0.05
Salad							
-ve	8	12.9	8	6.8	>0.05	2	1.3
+ve	54	87.1	110	93.2		0.5–8.8	0.7–2.7
						>0.05	>0.05

Item	Mothers of NTDs Patients		Control Mothers		P	Odd Ratio	Risk Ratio
	No.	%	No.	%			
Spinach							
-ve	8	12.9	42	35.6	<0.05	0.2681	0.696
+ve	54	87.1	76	64.4		0.1–0.9	0.53–0.9
						<0.05	<0.05
Tomato							
-ve	2	3.2	6	5.1	>0.05	0.62	0.86
+ve	60	96.8	112	94.9		0.1–6.2	0.5–1.6
						>0.05	>0.05
Zucchini							
-ve	16	25.8	36	30.5	>0.05	0.79	0.92
+ve	46	74.2	82	69.5		0.3–2.1	0.7–1.3
						>0.05	>0.05
Apple							
-ve	10	16.1	16	13.6	>0.05	1.2	1.1
+ve	52	83.9	102	86.4		0.4–4.1	0.7–1.7
						>0.05	>0.05
Orange							
-ve	26	41.9	90	76.3	<0.002	0.2247	0.5639
+ve	36	58.1	28	23.7		0.1–0.6	0.4–0.9
						<0.002	<0.002
Mango							
-ve	20	32.3	82	69.5	<0.001	0.2091	0.5741
+ve	42	67.7	36	30.5		0.1–0.5	0.4–0.8
						<0.001	<0.001
Coriander							
-ve	40	64.5	52	44.1	>0.05	2.3077	1.3269
+ve	22	35.5	66	55.9		0.9–5.7	0.9–1.8
						<0.05	<0.05
Cowpeas							
-ve	6	9.7	4	0	>0.05	3.0536	1.6765
+ve	56	90.3	114	100		0.48–19.3	0.6–5
						>0.05	>0.05
Greenpeas							
-ve	16	25.8	36	30.5	>0.05	0.7923	0.9253
+ve	46	74.2	82	69.5		0.3–2.1	0.7–1.3
						>0.05	>0.05
Artichoke							
-ve	52	83.9	108	91.5	>0.05	0.4	0.7
+ve	10	16.1	10	8.5		0.1–1.8	0.4–1.9
						>0.05	>0.05
Eggplant							
-ve	6	9.7	20	16.9	>0.05	0.525	0.8273
+ve	56	90.3	98	83.1		0.1–2.1	0.6–1.2
						>0.05	>0.05

NTDs = neural tube defects.

* Baladibread is traditional Egyptian bread made essentially from whole wheat.

† Taamiya is a traditional Egyptian food made of peeled fava beans mixed with green leafy vegetables and fried in vegetable oil.

was 52.3%. Despite being insignificant, it also points to the importance of combined effects, eventually in combination with the observed long cooking time and its role in the NTDs group.

One surprising, interesting finding in our study was that consumption of fava beans, cauliflower, spinach, and mango were observed more commonly in the NTDs group with mean odd ratios for NTDs of 0.13, 0.3, 0.27, and 0.21, respectively.

TABLE 4. Multivariate Regression for NTDs Determinants

Item	R	R ²	P
Residency	0.5821	33.9	<0.001
Education	-0.4972	24.7	<0.001
Family relation	0.4584	21.0	<0.01
Food preparation	0.4486	20.0	<0.01
Weight	0.4461	19.9	<0.01
Maternal DM	0.3967	15.7	<0.05
Mango	0.3741	14.0	=0.05

DM = diabetes mellitus, NTDs = neural tube defects.

That means that 1 in every 7, 1 in every 3, 1 in every 3, and 1 in every 4 mothers, respectively, will deliver a neonate with NTDs when eating these foods (Table 3). On the other hand, intake of orange and coriander was found to be associated with a lower NTD-risk (odds ratios 0.22 and 2.3, respectively, that is 1 in every 4 and 2 of every 3 neonates will be protected if their mothers ate these foods). Multicenter studies with longer duration should be carried out for deeper insight into these food peculiarities.

We did not find another study reporting the relationship between long cooking on the stability of folic acid in food. However, our study found higher prevalence of NTDs in the offspring of mothers with history of long food cooking.

Despite the pivotal role of genetic factors in the development of NTDs,^{35–37} it was beyond the scope of the present study.

One limitation in the present study is the possibility of the recall bias of the retrospective nutritional survey. Another limitation is that we were unable to collect information about income of the participants' families due to constraints related to respondent burden.

CONCLUSION

NTDs' occurrence is an environmentally multifactorial outcome resulting from the interaction of many variables which separately and by themselves could not be individually the direct causes of NTDs. These factors are different from country to another. The protective effect of folic acid intake against NTDs may depend on the synergism of those different socioeconomic and environmental factors. In Egypt, females especially the medium-educated who live in urban areas should be targeted towards promoting healthy lifestyle as well as adequate periconceptional supplementation of folic acid as a cornerstone in preventing NTDs.

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