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RESEARCH ARTICLE

RELATIONSHIP OF HEAVY METAL IN DIABETES AND NON-DIABETIC FOOT ULCER PATIENTS.

Prasanta Kumar Bhattacharyya[1], Priyanka Biswas[2], Debarshi Jana[3], Jayanta Ranjan Mukherjee[1] and Madhusanta De[2].

1. KPC Medical College & Hospital, Kolkata.
2. Ramakrishna Mission SevaPratishthan, Kolkata.
3. Institute of Post-Graduate Medical Education and Research, A.J.C. Bose Road, Kolkata-700020, West Bengal, India.

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Abstract

Diabetic foot ulcer is a major complication of diabetes mellitus, and probably the major component of the diabetic foot. The aim of our study was to find out any relationship of heavy metals like Arsenic, Cadmium, Mercury, Lead, Chromium, Barium, Cobalt, Caesium
We found that mean arsenic of Type2 DM with Foot Ulcer patients had significantly higher than others. Mean cadmium level had significantly lower in Type2 DM with Foot Ulcer than Healthy Control (t=3.5689). It may conclude that mercury level had lower in diabetic patients. Mean lead level had significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=2.3510). T-test showed that mean lead of Type2 DM with Foot Ulcer patients had significantly higher than others. Mean chromium level had significantly higher in Type2 DM with Foot Ulcer than others but that was not statistically significant. T-test showed that mean barium of Type2 DM without foot ulcer patients had significantly higher than others. Mean cobalt, caesium and selenium level had significantly higher in Type2 DM with Foot Ulcer than others but that was not statistically significant.
It can be suggested that toxic metals such as arsenic, cadmium, mercury, lead, chromium, barium, cobalt, caesium and selenium may have a role to induce foot ulcer in diabetic subjects.

Introduction:-

Diabetic foot ulcer is a major complication of diabetes mellitus, and probably the major component of the diabetic foot. Wound healing is an innate mechanism of action that works reliably most of the time. A key feature of wound healing is stepwise repair of lost extracellular matrix (ECM) that forms the largest component of the dermal skin layer.[1]But in some cases, certain disorders or physiological insult disturbs the wound healing process. Diabetes mellitus is one such metabolic disorder that impedes the normal steps of the wound healing process. Many studies show a prolonged inflammatory phase in diabetic wounds, which causes a delay in the formation of mature granulation tissue and a parallel reduction in wound tensile strength.[2]

Corresponding Author:-Prasanta Kumar Bhattacharyya.
Address**:**-KPC Medical College & Hospital, Kolkata.

Treatment of diabetic foot ulcers should include: blood sugar control, removal of dead tissue from the wound, wound dressings, and removing pressure from the wound through techniques such as total contact casting. Surgery in some cases may improve outcomes.[3] Hyperbaric oxygen therapy may also help but is expensive.[3]

It occurs in 15% of people with diabetes,[4] and precedes 84% of all diabetes-related lower-leg amputations.[5]

Toxic heavy metals such as arsenic (As), lead (Pb), and mercury (Hg) are systemic toxicants that are hazardous to human health. However, as these elements are increasing in the environment due to fast urbanization, industrialization, and chemicalized agricultural activities, accumulation of the same in human body anywhere in the world is quite interesting to global assessment of environment quality. In this connection, random examination of blood samples of human population in Kerala, South India, was carried out to assess the threat of heavy metal contamination to humans in this part of the globe, especially in relation to the amount of such metals in food and other environmental samples. Except pure vegetarians, people of Kerala consume rice as the staple food with a lot of fish. Therefore, the amount of these three heavy metals in drinking water, fish, rice, and paddy soils was done. Heavy metals in the blood were examined in relation to age, gender, and dietary habits such as frequency of fish eating or vegetarianism. Influence of dental amalgam fillings on blood mercury levels was also analyzed. Quantitative assessment of metals in samples was done by inductively coupled plasma-mass spectrometry (ICP-MS). The levels of arsenic, lead, and mercury were found well below the reference values, though diet seemed to pull them up as the amount of metals in blood showed significant differences between vegetarians and non-vegetarians. Evidence to the influence of dental amalgam fillings on blood mercury levels could not be established with the present samples.

To Study Role of Environmental Factors:-
1. Trace elements – Selenium.
2. Heavy metals – Arsenic, Cadmium, Mercurry, Lead, Chromium, Barium, Cobalt, Caesium.

Materials And Methods:-

Inclusion Criteria
1. Vascular foot ulcers
2. Neuropathic foot ulcers
3. Infective foot ulcers
4. Healthy Control.

Exclusion Criteria
1. Traumatic Ulcers
2. Steroid Induced Ulcers
3. Malignant Ulcers
4. Radiation Ulcers
5. Skin diseases

Sample Design
1. Healthy Control, 50
2. Diabetic population with foot ulcer, 50
3. Diabetic population without foot ulcer, 50
4. Non-diabetic population with foot ulcer, 50

Study group:
1. Healthy Control, 50 persons
2. Diabetic population with foot ulcer, 50patients
3. Diabetic population without foot ulcer, 50patients
4. Non-diabetic population with foot ulcer, 50patients

Statistical analysis:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS (version 24.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Two-sample t-tests for a difference in mean involved independent samples or unpaired samples. Paired t-tests were a form of blocking and had greater power than unpaired tests. One-way analysis of variance (one-way ANOVA) was a technique used to compare means of three or more samples for numerical data (using the F distribution). A chi-squared test ($\chi 2$ test) was any statistical hypothesis test wherein the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true. Without other qualification, 'chi-squared test' often is used as short for Pearson's chi-squared test. Unpaired proportions were compared by Chi-square test or Fischer's exact test, as appropriate.

p-value ≤ 0.05 was considered for statistically significant.

Results:-

We found that in type2 DM with foot ulcer, the mean age (mean±s.d.) of patients was 4.6200± 10.8438 years. In non-diabetic foot ulcer, the mean age (mean±s.d.) of patients was 42.3600± 13.4661 years. In type2 DM without foot ulcer, the mean age (mean±s.d.) of patients was 50.5000± 11.3986 years. In Healthy Control, the mean age (mean±s.d.) of patients was 45.1800± 15.2472 years. Distribution of mean age vs. group was statistically significant ($p<0.0001$). It was found that association of sex vs. group was statistically significant ($p<0.0001$).

We found that in type2 DM with foot ulcer, the mean arsenic (mean±s.d.) of patients was 3.9860 ± 1.3040 µg/l. In non-diabetic foot ulcer, the mean arsenic (mean±s.d.) of patients was 2.5064 ± .7588 µg/l. In type2 DM without foot ulcer, the mean arsenic (mean±s.d.) of patients was 1.3776 ± .1388 µg/l. In Healthy Control, the mean arsenic (mean±s.d.) of patients was 1.3024 ± .2364 µg/l. Distribution of mean arsenic vs. group was statistically significant ($p<0.0001$).

It was found that in type2 DM with foot ulcer, the mean cadmium (mean±s.d.) of patients was .5074 ± .2551µg/l. In non diabetic foot ulcer, the mean cadmium (mean±s.d.) of patients was .6814 ± .2000 µg/l. In type2 DM without foot ulcer, the mean cadmium (mean±s.d.) of patients was .8140 ± .3451 µg/l. In Healthy Control, the mean cadmium (mean±s.d.) of patients was .6728 ± .2057 µg/l. Distribution of mean cadmium vs. group was statistically significant ($p<0.0001$).

We found that in type2 DM with foot ulcer, the mean mercury (mean±s.d.) of patients was 1.1340 ± .6895 µg/l. In non diabetic foot ulcer, the mean mercury (mean±s.d.) of patients was 1.3304 ± .5559 µg/l. In type2 DM without foot ulcer, the mean mercury (mean±s.d.) of patients was 1.1232 ± .4659 µg/l. In Healthy Control, the mean mercury (mean±s.d.) of patients was 1.3974 ± .5635 µg/l. Distribution of mean mercury vs. group was statistically significant ($p=0.0354$).

We found that in type2 DM with foot ulcer, the mean lead (mean±s.d.) of patients was 36.8300 ± 19.4565 µg/l. In non-diabetic foot ulcer, the mean lead (mean±s.d.) of patients was 31.9012 ± 17.2549 µg/l. In type2 DM without foot ulcer, the mean lead (mean±s.d.) of patients was 30.0516 ± 4.5093 µg/l. In Healthy Control, the mean lead (mean±s.d.) of patients was 28.5864 ± 15.3693 µg/l. Distribution of mean lead vs. group was statistically significant ($p=0.0432$).

It was found that in type2 DM with foot ulcer, the mean chromium (mean±s.d.) of patients was 2.0360 ± 7.5584 µg/l. In non-diabetic foot ulcer, the mean chromium (mean±s.d.) of patients was 1.3420 ± 2.4775 µg/l. In type2 DM without foot ulcer, the mean chromium (mean±s.d.) of patients was .6500 ± .2244 µg/l. In Healthy Control, the mean chromium (mean±s.d.) of patients was .6150 ± .2180 µg/l. Distribution of mean chromium vs. group was not statistically significant ($p=0.2391$).

It was found that in type2 DM with foot ulcer, the mean barium (mean±s.d.) of patients was 26.0608 ± 11.2050 µg/l. In non diabetic foot ulcer, the mean barium (mean±s.d.) of patients was 11.6326 ± 7.5489 µg/l. In type2 DM without foot ulcer, the mean barium (mean±s.d.) of patients was 18.4122 ± 9.4575µg/l. In Healthy Control, the mean barium (mean±s.d.) of patients was 11.5628 ± 8.0150 µg/l. Distribution of mean barium vs. group was statistically significant ($p<0.0001$).

We found that in type2 DM with foot ulcer, the mean cobalt (mean±s.d.) of patients was .8302 ± 2.6928 µg/l. In non diabetic foot ulcer, the mean cobalt (mean±s.d.) of patients was .6194 ± .4213 µg/l. In type2 DM without foot ulcer, the mean cobalt (mean±s.d.) of patients was .4240 ± .1062 µg/l. In Healthy Control, the mean cobalt (mean±s.d.) of patients was .3580 ± .1056 µg/l. Distribution of mean cobalt vs. group was not statistically significant (p=0.3129).

It was found that in type2 DM with foot ulcer, the mean caesium (mean±s.d.) of patients was 2.1116 ± .3510 µg/l. In non diabetic foot ulcer, the mean caesium (mean±s.d.) of patients was 1.5332 ± .3032 µg/l. In type2 DM without foot ulcer, the mean caesium (mean±s.d.) of patients was 1.6418 ± .9908 µg/l. In Healthy Control, the mean caesium(mean±s.d.) of patients was 1.5224 ± .3213 µg/l. Distribution of mean caesium vs. group was statistically significant (p<0.0001).

We found that in type2 DM with foot ulcer, the mean selenium (mean±s.d.) of patients was 180.5586 ± 28.8920 µg/l. In non-diabetic foot ulcer, the mean selenium (mean±s.d.) of patients was 170.7362 ± 26.7228 µg/l. In type2 DM without foot ulcer, the mean selenium (mean±s.d.) of patients was 150.6476 ± 37.0882 µg/l. In Healthy Control, the mean selenium (mean±s.d.) of patients was 120.6164 ± 22.9464 µg/l. Distribution of mean selenium vs. group was statistically significant (p<0.0001).

We found that mean arsenic level had significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=14.3184). T-test showed that mean arsenic of Type2 DM with Foot Ulcer patients had significantly higher than others. Mean cadmium level had significantly lower in Type2 DM with Foot Ulcer than Healthy Control (t=3.5689). T-test showed that mean cadmium of Type2 DM with Foot Ulcer patients had significantly lower than others. Mean mercury level had significantly lower in Type2 DM with Foot Ulcer and Type2 DM without foot ulcer (t=2.0917and 2.6518 respectively).It may conclude that mercury level had lower in diabetic patients. Mean lead level had significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=2.3510). T-test showed that mean lead of Type2 DM with Foot Ulcer patients had significantly higher than others.

Mean chromium level had significantly higher in Type2 DM with Foot Ulcer than others but that was not statistically significant. Mean barium level had significantly higher in Type2 DM without foot ulcer than Healthy Control (t=7.4414). T-test showed that mean barium of Type2 DM without foot ulcer patients had significantly higher than others. Mean cobalt level had significantly higher in Type2 DM with Foot Ulcer than others but that was not statistically significant. Mean caesium level had significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=8.7551). T-test showed that mean caesium of Type2 DM with Foot Ulcer patients had significantly higher than others. Mean selenium level had significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=11.4880). T-test showed that mean selenium of Type2 DM with Foot Ulcer patients had significantly higher than others.

Discussion:-

Diabetic foot ulcer is the common dreadful complication of diabetes mellitus. The lifetime prevalence of foot ulceration is about 15%. [6] Macro and microvascular involvement and neuropathy plays a major role in the pathophysiology of diabetic foot ulcers. [7] According to the Diabetes Atlas 2013 published by the International Diabetes Federation, the number of people with diabetes in India currently is 65.1 million, which is expected to rise to 142.7 million by 2035. [8] Mean age of the study population was 51 years, which is in par with the previous studies in India. [9]

We found that mean age was higher in type2 DM with foot ulcer patients than others and that was statistically significant (p<0.0001).Present study found that male had more prevalence in Type2 DM with Foot Ulcer and it was statistically significant (p<0.0001). In type2 DM with foot ulcer, higher number of patients 16(32.0%) were house wives. In non-diabetic foot ulcer, higher number of patients 28(56.0%) were house wives. In type2 DM without foot ulcer, higher number of patients 26(52.0%) were house wives. In healthy control, higher number of patients 29(58.0%) were house wives. Association of occupation vs. group was not statistically significant (p=0.0002).
Some metals (e.g. magnesium) are known as macro-metals and are found in high amount in the body tissues, therefore they are also called macro-nutrients.[10] At least 100 mg of each macro-nutrient is required in the daily diet [11]. In contrast, some metals e.g. copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn), chromium (Cr) etc. are needed in the body in very small amounts, less than 100 parts per million (ppm), hence, these are called trace elements or micro-nutrients [12]. Metals are involved in a range of physiological processes such as prosthetic groups of many proteins, water balance, cofactors of many enzymes etc.[13] Several metals function as part of

proteins/enzymes as metalloproteinase/metalloenzymes.[14] Such proteins without metal containing prosthetic groups are unable to perform their physiological functions.[15] The regulation of various metallic contents in the body is pre-requisite for their proper functioning.[16] Metals enable the muscles to contract or relax, and also transmit impulses through the nerves. Most metals are available in the soluble salt forms, which regulate the composition of biofluids. The proper metabolic functioning of the trace elements depends on their normal levels in various body tissues.[17] Due to the diversified metabolic characteristics and functions; various metals such as Mg, Zn, Cr, Fe, Mn and Cu are considered as essential for normal human health. Several studies have reported that the imbalance of some essential metals might adversely affect pancreatic islet and cause development of diabetes.[18]It is also manifested that some reactive oxygen species (ROS) are produced during diabetes due to imbalance of essential metals. This oxidative stress might decrease the insulin gene promoter activity and mRNA expression in pancreatic islet cells due to hyperglycemic condition.[19-20]

We found that mean arsenic level had significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=14.3184). T-test showed that mean arsenic of Type2 DM with Foot Ulcer patients had significantly higher than others.

It was found that mean cadmium level was significantly lower in Type2 DM with Foot Ulcer than Healthy Control (t=3.5689). T-test showed that mean cadmium of Type2 DM with Foot Ulcer patients was significantly lower than others. Mean mercury level was significantly lower in Type2 DM with Foot Ulcer and Type2 DM without foot ulcer (t=2.0917and 2.6518 respectively).It may conclude that mercury level was lower in diabetic patients. Mean lead level wassignificantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=2.3510). T-test showed that mean lead of Type2 DM with Foot Ulcer patients were significantly higher than others.Mean chromium level was significantly higher in Type2 DM with Foot Ulcer than others but that was not statistically significant.

We found that mean barium level was significantly higher in Type2 DM without foot ulcer than Healthy Control (t=7.4414). T-test showed that mean barium of Type2 DM without foot ulcer patients was significantly higher than others.Mean cobalt level was significantly higher in Type2 DM with Foot Ulcer than others but that was not statistically significant. Mean caesiumlevel was significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=8.7551). T-test showed that mean caesium of Type2 DM with Foot Ulcer patients were significantly higher than others.Mean selenium level was significantly higher in Type2 DM with Foot Ulcer than Healthy Control (t=11.4880). T-test showed that mean selenium of Type2 DM with Foot Ulcer patients was significantly higher than others.

Conclusion:-

From our study on derangement of metals in diabetes, it could be concluded that normal levels of essential metals are disturbed in T2D patients. It can be suggested that toxic metals such as arsenic, cadmium, mercury, lead, chromium, barium, cobalt, caesium and selenium may have a role to induce foot ulcer in diabetic subjects.

1. Heavy metal toxicity may have significance in the occurrence of DFU.
2. Future work can be done to find the causative effect of heavy metals in DFU.
3. Pollution from soil, water, food, air may have some action.
4. Regional variation between cities and villages and varied geographical samples may direct to the aetiopathogenesis of this dreaded DFU.

Table1:- Distribution of mean heavy metals in four groups

	Group	Number	Mean	SD	Minimum	Maximum	Median	p-value
ARSENIC µg/l	Type2 DM with foot ulcer	50	3.9860	1.3040	2.1000	5.8900	4.2500	<0.0001
	Non diabetic foot ulcer	50	2.5064	.7588	1.2500	5.2300	2.6700	
	Type2 DM without foot ulcer	50	1.3776	.1388	1.2200	1.6400	1.3150	
	Healthy Control	50	1.3024	.2364	1.0100	1.7100	1.2350	
CADMIUM µg/l	Type2 DM with foot ulcer	50	.5074	.2551	0.2000	0.9700	0.4550	<0.0001
	Non diabetic foot	50	.6814	.2000	0.4000	0.9400	0.7800	

	ulcer							
	Type2 DM without foot ulcer	50	.8140	.3451	0.2000	1.6200	0.9300	
	Healthy Control	50	.6728	.2057	0.4500	0.9400	0.7800	
MERCURY µg/l	Type2 DM with foot ulcer	50	1.1340	.6895	0.4500	2.6400	0.9000	0.0354
	Non diabetic foot ulcer	50	1.3304	.5559	0.5000	1.8900	1.4500	
	Type2 DM without foot ulcer	50	1.1232	.4659	0.4500	2.6400	1.1900	
	Healthy Control	50	1.3974	.5635	0.5000	1.8900	1.4500	

	Group	Number	Mean	SD	Minimum	Maximum	Median	p-value
LEAD µg/l	Type2 DM with foot ulcer	50	36.8300	19.4565	1.4800	54.3800	51.8300	0.0432
	Non diabetic foot ulcer	50	31.9012	17.2549	13.3900	58.0600	30.2300	
	Type2 DM without foot ulcer	50	30.0516	4.5093	13.7200	42.5700	30.2500	
	Healthy Control	50	28.5864	15.3693	13.3900	53.3300	24.1800	
CHROMIUM µg/l	Type2 DM with foot ulcer	50	2.0360	7.5584	0.7400	54.3800	0.9500	0.2391
	Non diabetic foot ulcer	50	1.3420	2.4775	0.5700	11.0200	0.6200	
	Type2 DM without foot ulcer	50	.6500	.2244	0.3600	1.0000	0.5350	
	Healthy Control	50	.6150	.2180	0.5200	2.1000	0.5900	
BARIUM µg/l	Type2 DM with foot ulcer	50	26.0608	11.2050	0.7400	38.2600	32.3600	<0.0001
	Non diabetic foot ulcer	50	11.6326	7.5489	2.2500	23.4600	12.3300	
	Type2 DM without foot ulcer	50	18.4122	9.4575	2.3800	28.8200	19.5900	
	Healthy Control	50	11.5628	8.0150	2.2500	23.4600	10.3700	
COBALT µg/l	Type2 DM with foot ulcer	50	.8302	2.6928	0.3600	19.4800	0.3700	0.3129
	Non diabetic foot ulcer	50	.6194	.4213	0.2300	1.2300	0.3800	
	Type2 DM without foot ulcer	50	.4240	.1062	0.2400	0.5300	0.4500	
	Healthy Control	50	.3580	.1056	0.2300	0.5100	0.3450	

	Group	Number	Mean	SD	Minimum	Maximum	Median	p-value

CAESIUM µg/l	Type2 DM with foot ulcer	50	2.1116	.3510	0.5700	2.4100	2.2500	<0.0001
	Non diabetic foot ulcer	50	1.5332	.3032	1.1700	2.0400	1.5300	
	Type2 DM without foot ulcer	50	1.6418	.9908	0.9700	3.2100	1.0400	
	Healthy Control	50	1.5224	.3213	1.1700	2.0400	1.3900	
SELENIUM µg/l	Type2 DM with foot ulcer	50	180.5586	28.8920	117.0500	200.9600	190.6200	<0.0001
	Non diabetic foot ulcer	50	170.7362	26.7228	110.2200	200.8800	160.5600	
	Type2 DM without foot ulcer	50	150.6476	37.0882	97.0900	200.3600	129.7400	
	Healthy Control	50	120.6164	22.9464	84.2900	200.4500	117.0500	

Table 2:- Distribution of heavy metals in four groups

		T Statistic	**P-value**
ARSENIC µg/l	Type2 DM with Foot Ulcer vs Healthy Control	14.3184	<0.0001
	Type2 DM without Foot Ulcer vs Healthy Control	1.9399	0.0553
	Non diabetic foot ulcer vs Healthy Control	10.7116	<0.0001
CADMIUM µg/l	Type2 DM with Foot Ulcer vs Healthy Control	3.5689	<0.0001
	Type2 DM without Foot Ulcer vs Healthy Control	2.4852	0.0146
	Non diabetic foot ulcer vs Healthy Control	0.2120	0.8326
MERCURY µg/l	Type2 DM with Foot Ulcer vs Healthy Control	2.0917	0.0391
	Type2 DM without Foot Ulcer vs Healthy Control	2.6518	0.0093
	Non diabetic foot ulcer vs Healthy Control	0.5985	0.5509
LEAD µg/l	Type2 DM with Foot Ulcer vs Healthy Control	2.3510	0.0207
	Type2 DM without Foot Ulcer vs Healthy Control	0.6468	0.5192
	Non diabetic foot ulcer vs Healthy Control	1.0144	0.3129
CHROMIUM µg/l	Type2 DM with Foot Ulcer vs Healthy Control	1.3288	0.1870
	Type2 DM without Foot Ulcer vs Healthy Control	0.7911	0.4308
	Non diabetic foot ulcer vs Healthy Control	2.0670	0.0414
BARIUM µg/l	Type2 DM with Foot Ulcer vs Healthy Control	7.4414	<0.0001
	Type2 DM without Foot Ulcer vs Healthy Control	3.9068	0.0002
	Non diabetic foot ulcer vs Healthy Control	0.0448	0.9643
COBALT µg/l	Type2 DM with Foot Ulcer vs Healthy Control	1.2390	0.2183
	Type2 DM without Foot Ulcer vs Healthy Control	3.1151	0.0024
	Non diabetic foot ulcer vs Healthy Control	4.2554	<0.0001
CAESIUM µg/l	Type2 DM with Foot Ulcer vs Healthy Control	8.7551	<0.0001
	Type2 DM without Foot Ulcer vs Healthy Control	0.8106	0.4196
	Non diabetic foot ulcer vs Healthy Control	0.1729	0.8631
SELENIUM µg/l	Type2 DM with Foot Ulcer vs Healthy Control	11.4880	<0.0001
	Type2 DM without Foot Ulcer vs Healthy Control	4.8690	<0.0001
	Non diabetic foot ulcer vs Healthy Control	10.0617	<0.0001

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