Assessment of Transthyretin Serum Level in Children Using Mobile Phones

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Abstract

Purpose of the study:to assess whether the use of mobile phones by children would affect the Full-scale IQ and / or the concentrations of serum Transthyretin (TTR) level which is a marker of the blood brain barrier integrity.
Methods:ninety children, considered as a case if they own a mobile phone with regular use in the last month, they are classified into 3 groups according to the age:**group I**: includes 30 children aged 4 – 6 years, **group II**: includes 30 children aged 6 – 13 years, and **group III:** includes 30 children aged 13 – 18 years. Ninety children were age matched according to the previous classification, they were considered as non-users when they are not owing mobile phone and even not using it regularly in the last month from any nearby source.
Full-scale Intelligence Quotient (IQ) and serum Transthyretin levels were done to all studied groups.
Results: transthyretin serum levels were significantly higher among users than non-users in total age, 13-18, and 6-13 age groups. There were significant effects of MPRF exposure on serum transthyretin levels in both total and 13-18 age groups (p value =0.003 and <0.001 respectively). MPRF exposure has also a significant effect on the Full-scale IQ in only 13-18 age group (p= 0.05).
Conclusion: Raised serum Transthyretin level (which denote blood brain barrier leak) observed with longer duration of mobile phone use and associated with decrease in full-scale IQ.
Key words: mobile phones, IQ, Transthyretin, children, brain integrity.
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Introduction

Transthyretin (TTR),originally called prealbumin, is a transport protein in the plasma and cerebrospinal fluid that transports the thyroid hormone thyroxine (T_4) and retinol to the liver(1).

Transthyretin is responsible for the transport of thyroxin and retinol binding protein complex. It is also involved in cardiovascular diseases, Alzheimer's disease, diabetes, amyloidosis, psychological, cognitive disorders, polyneuropathy, and obesity(2).

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Recently, transthyretin isproved to be associated with many biological functions that are associated eighter directly or indirectly with oxidative stress which is included in many human diseases(3).

Its major sites of synthesis are the liver, the epithelial cells of the choroid plexus (CP) that located in the ventricles, representing about 25% of the CSF protein, and the retinal pigment epithelium(4).

There is ongoing concern about possible health and/or developmental effects of children's exposure to radiofrequency electromagnetic fields (RF-EMF) (5).Higher conductivity of their brain tissues, greater RF penetration due to differences in the size, shape, water content and tissue distribution of the brain in children and finally the fact that children have longer lifetime exposure than adults. These factors make the specific absorption rate (SAR) of RF radiation in the children brain is higher than adults(6).

Dysfunction of the blood-brain barrier (BBB) is one such effect that has long been debated. A barrier that has received much less attention, though it also serves to maintain brain homeostasis by separating the central nervous system from the blood stream(7).

Aim of the work

To assess whether the use of mobile phones by children would affect the Full-scale Intelligence Quotient (IQ)and / or the concentrations of serum Transthyretin (TTR) level which is a marker of the blood brain barrier integrity.

Subjects and methods

This retrospective case-control study was conducted on the relative of patients attending pediatrics outpatient clinic at the Minia university hospital, Egypt during the period from June 2020 to December 2021. One hundred eighty children of different socioeconomic status were included in our study. They were divided into; **patient group (cases):**ninety mobile users children (they own a mobile phone and use it regularly for calling in the last month). Patients groups weresubdivided into 3 subgroups according to the age:

Group I: included 30 children aged 4 – 6 yrs.

Group II: included 30 children aged 6 – 13 yrs.

Group III: included 30 children aged 13 – 18 yrs.

Control group: ninety children, non-users when they are not owing mobile phone and even not using it regularly in the last month from any nearby source i.e., parents or older siblings. Both groups were age and sex matched.

All children from both sex and their ages ranged from 4 to 18 years with different socioeconomic status were included in our study.

We excluded children aged below 4 and above 18 years, children with central nervous system pathology or multi-systemic disease known to affect central nervous system, children with psychiatric disorders, children suffering from malnutrition, liver disease, any chronic illness or receiving any medication known to affect cognitive function. Children living or going to school nearby mobile phone base station (MPBSTs) were also excluded from our study.

Written informed consents were taken from parents of all enrolled children after clarifying the aim and all steps of the study.Ethics approval was obtained from the Faculty of Medicine Research Ethical Committee, Minia University. The study was in accordancewith tenets of the Declaration of Helsinki. Informed consents were obtained from legal guardians of all subjects.

Methods

All enrolled children were subjected to full history taking, with emphasis on age, residence, level of education.RF-EMF exposure was measured according to the mobile phone use by a questionnaire which was answered by the

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children themselves in the presence of their parents, were categorized into duration of calls per minutes, frequency of calls per day, total number of voice calls (made and received) weekly, and the duration of mobile phone usage by years. Laterality (Side of the head that frequently used during the active call) right, left or both sides. Use of hand free device during the active call operation.

Clinical examination

General and systemic examination were done for all subjects to exclude any medical disease which may affect the cognitive functions.Full-scale Intelligence Quotient (IQ) was done to all included children.

Laboratory measure of transthyretin

Blood sampling protocol: two ml of venous blood were withdrawn from all children shared in that study by using a disposable plastic syringe after sterilization of skin with isopropyl alcohol (70%) swaps. The blood sample was collected in gel separator vacutainer tube, blood was left to clot in the incubator then centrifuged. The expressed serum was kept in -20◦C later to assay transthyretin. Serum transthyretin was assayed by EIA method (kit was supplied by Bioassay technology laboratory (BT LAB) cat no. EA0000Hu). Serum transthyretin level in healthy children is age dependent:14 - 30 mg/dL for children ages 1 to 6, 15 - 33 mg/dL for children ages 6 to 13, and 22 - 45 mg/dL for those ages 13 to 19 (8).

Statistical analysis

The data were coded, tabulated, and analyzed using statistical package for social sciences (SPSS), software version 25.

Mann Whitney test used for non-parametric quantitative data between the two groups and Qualitative data were expressed as numbers and percentages.

Chi-square test was applied to test the relationship between variables and Fisher's exact test for qualitative data between the two groups (if expected number per cell < 5) and Chi square test (if expected number per cell > 5).

Kruskal Wallis test for non-parametric quantitative data between the three age groups, followed by Mann Whitney test between each two groups.

Binary logistic regression analysis test was used for prediction of IQ Scales between mobile user and nonusers, odd ratio and confidence interval were calculated.

Simple logistic regression analysis of transthyeretin was used to predict the mobile use in children, odd ratio and confidence interval were calculated.

The level of significance was taken at (P value < 0.05).

Results

Our case-control study was conducted on 180 children. They were classified into:

The first subgroup includes 30 children aged from 4 – 6 yrs.

The second subgroup includes 30 children aged from 6 – 13 yrs.

Third subgroup includes 30 children aged from 13 – 18 yrs.

They were considered as a case if they owned a mobile phone and used it regularly during the last month.

Ninety children (Controls or nonusers) were age matched according to the previous age subgroups stratifications, they were considered non-users when they are not owing mobile phone and even not using it regularly at least in the last month from any nearby sources i.e., parents or older siblings. Serum transthyretin levelswere measured by blood sampling in all our studied children.

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Regarding demographic data, there was no significant difference between users and nonusers regarding age, sex, and residence. The type of schooleducation, private school was more prevalent between users (42.2%) than nonusers (25.6%), p value = 0.002.**(Table 1)**

There weresignificant differences between the users of different age groups with higher values in 13-18 age group and lowest values in the 4-6 age group regarding duration of call by minutes, number of call/days, number of call/weeks,and duration of use/year, P value <0.001.**(Table 2)**

Regarding full scale IQ, there was a significant difference between nonusers and users in only the 13-18 age subgroup, p value = 0.012 **(Table 3)**

Serum transthyretin levels were significantly higher among users than non-users in total age, 13-18, and 6-13 age groups. **(Table 4)**

There were significant effects of MPRF exposure on serum transthyretin levels in both total and 13-18 age groups (p value =0.003 and<0.001 respectively). MPRF exposure has also a significant effect on the Full-scale IQ in only 13-18 age group (p= 0.05).**(Table 5)**

Both total and 13-18 age groups, there were positive correlations between serum trasthyretin levels withboth the duration of calls by minutes (r= 0.403 and 0.384 with p<0.001and 0.036respectively) and the duration of use/years (r=0.778 and 0.798with p<0.001respectively). While there was positive correlation between serum trasthyretin levels withthe number of call/weeks in the total age group only (0.557, p <0.001)**(Table 6)**

Discussion

The number of mobile users worldwide was 7.1 billion in 2021, it is likely to rise to 7.26 billion by 2022. In 2025, the number of mobile users all over the world is suspectted to reach 7.49 billion(9).

Youngs are more vulnerable due to their still developing nervous systems and the potential for higher cumulative RF exposure(10). Differences in the tissue distribution, shape, size, and water content of the brain in children are likely to be responsible for the higher SAR and vulnerability of children(6)**.**

It is widely known that TTR has an important role in the central nervous system, especially in memory, cognition, psychological health, and emotion(3).

To the best of our knowledge, our study is one of very few studies that detect whether the use of mobile phones could affect the Full-scale IQ in children and detect its effect on BBB integrity via measuring the concentrations of serum TTR as a marker for alterations in CSF TTR.

In our study, we noticed that 13-18 age group had the highest values regarding duration of call by minutes, number of call/days, number of call/weeks, and duration of use/year. They also had lower level regarding full scale IQ.MPRF exposure has also a significant effect on the Full-scale IQ in only 13-18 age group.

A study done by ***Söderqvist et al. (2009)*** who concluded that the use of mobile phones in adults was significantly associated with higher TTR levels. This association was for longer periods of mobile phone use > 5 years and for more duration of call >15 minutes/call(11).Also, both***Sinha et al.*** and ***Wilmer et al***stated that mobile phone radiofrequency and other mobile technologies leads to impairment of cognition(12, 13).

Sage and Burgio, (2018) concluded that disruption of neural synchrony by RFR exposure may be the key factor in disrupted cognition(14).

However, ***Calvente et al., (2016***) examined 9–11 years old students and found that higher exposures were significantly associated with lower IQ scores. This difference can be explained by higher exposure to EMF-RF from other sources rather than mobile phones (MP) in their study as mobile phone base stations (MPBSTs), and personal devices as tablets,

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these EMF-RF sources were excluded in our study(15).

Ismail M stated that phones have negative effects on adolescents and concluded that devices could help individuals in quick access to information, but it is not an enough reason to risk the fact that it is causing a lot of havoc to the educational system(16).

Hazards on the brain of young children are of great concern, not only due to higher absorption than the brain of adults but also, their brain is growing rapidly, and thus more susceptible to insults especially glial cells(17).

Myelin provides some protection of neurons from RF and other neurotoxins. Myelin is thin in young brains and develops through the mid-twenties(18).Both lower myelin levels and higher water levels are responsible for greater RF energy absorption in young brains(19).

In our study, total, 13-18, and 6-13 age groups had higher serum transthyretin levels among users than non-users.
MPRF exposure had a significant effect on serum transthyretin levels in both total and 13-18 age groups.
This may be explained by the differences in mobile phone use pattern between different age groups with highest exposure values were in the oldest age group. This was confirmed by correlating the exposure items (duration of calls minutes/call and duration of use/years) with serum transthyertin levels which showed significant positive correlation in the total and 13-18 age groups.
These results agree with the study done by ***Söderqvist et al., (2009)*** who concluded that the use of mobile phones was significantly associated with higher TTR levels in adults(11).
Söderqvist et al., (2015) concluded that there is no short term effects of MPRF on the serum levels of TTR and BBB permeability(20).

These previous two studies are consistent with our results that conclude that organic damage to BBB takes a long time to develop, this explains why those effects appeared only in the highest age group with more duration of exposure.
On explaining how RF could raise the TTR levels, there are at least a couple of potential mechanisms. One is dysfunction of the BCSFB leading to increased leakage or turnover of TTR in the choroid plexus; the other is up-regulation of the TTR gene in epithelial cells by the emitted MPRF(21).
Eberhardt et al., (2008) reported that exposure to EMR causes structural damage to the brain, and presence of shrunken neurons in the pyramidal cellresulting from albumin leakage from the BBB(22).

According to ***Fong and Vieira, (2013)*** TTR could be related to oxidative stress, as its level correlates well with reactive oxygen species (ROS) or reactive nitrogen species (RNS)(23).

oxidative stress is a main cause of Alzheimer's disease (AD) and other neurodegenerative diseases(24). TTR level is up regulated in patients with neurodegenerative disorders, wherein oxidative stress is the common cause of the pathophysiology (25, 3).

Conclusion

Raised serum Transthyretin level (which denote blood brain barrier leak) observed with longer duration of mobile phone use and associated with decrease in full-scale IQ.

Contribution to the field

The number of mobile phones users is continuously increasing. There is ongoing concern about possible health and/or developmental effects of children's exposure to mobile phones. In our research, we study the effect of mobile use on the full- scale IQ and on Transthyretin serum level (TTR) as a marker of blood brain barrier integrity.

Data availability

The datasets can be shared from the corresponding author on reasonable request.

Ethics Statement

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Ethics approval was obtained from the Faculty of Medicine Research Ethical Committee, Minia University. The study was in accordancewith tenets of the Declaration of Helsinki. Informed consents were obtained from legal guardians of all subjects.

Author contributions

Asmaa N. Mostafa, Reem A. Abdel Aziz, Doaa M Mahrous, and Hend M Moness conceived the study, carried out its design, coordinated the implementation, helped to perform the statistical analysis, and drafted the manuscript. All authors designed the study.AN and RA participated in the analysis and interpretation of data. RA and DM revised the statistics and final draft of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest statement

All authors declare that there is no conflict of interest.

List of abbreviations

- AD: Alzheimer's disease.
- BBB: Blood-brain barrier.
- BT LAB: Bioassay Technology Laboratory.
- CP: Choroid Plexus.
- CSF: Cerebrospinal Fluid.
- Full-scale IQ: Full-scale Intelligence Quotient.
- MP: Mobile Phones.
- MPBSTs: Mobile Phone Base Stations.
- MPRF: Mobile Phones Radio Frequency.
- RF-EMF: RadioFrequency Electromagnetic Fields.
- RNS: Reactive Nitrogen =Species.
- ROS: Reactive Oxygen Species.
- SAR: Specific Absorption Rate.
- SPSS: Statistical Package for Social Sciences.
- T4: Thyroxine.
- TTR: serum Transthyretin.

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Tables

Table (1) Demographic data of the studied children

All age groups			**Nonuser**	**User**	**P value**
			N=90	**N=90**	
Age (years)		*Median / IQR*	9.4/ (5.5-13.2)	9.7 / (5.7-13.2)	*0.326*
Age group	*13-18*	*Median / IQR*	13.6 / (13.2-14)	14 / (13.2-17.2)	*0.169*
	6-13	*Median / IQR*	9.4/ (7.7-11)	9.7 / (8.6-10.2)	*0.690*
	4-6	*Median / IQR*	5 / (4.3-5.5)	5.5 / (5.1-5.7)	*0.091*
Sex		*Male* *Female*	49(54.4%) 41(45.6%)	47(52.2%) 43(47.8%)	*0.765*
Residence		*Urban* *Rural*	74(82.2%) 16(17.8%)	74(82.2%) 16(17.8%)	*1*
School		*Governmental* *Private*	67(74.4%) 23(25.6%)	52(57.8%) 38(42.2%)	***0.002***

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Table (2) Comparison between different age groups users regarding pattern of mobile phone use

Users group		Age groups			P value
		13-18 years	6-13 years	4-6 years	
		N=30	N=30	N=30	
Duration of call by minutes	*Median / IQR*	3.5 / (2-6.3)	2.5 / (2-5)	1.5 / (1-2)	***<0.001***
Number of call/days	*Median / IQR*	4 / (3-6)	3 / (2-4)	1 / (1-2)	***<0.001***
Number of call/weeks	*Median / IQR*	28 / (20-36.3)	15.5 / (9.8-28)	6 / (4-8.3)	***<0.001***
Duration of use /year	*Median / IQR*	2.8 / (2-4)	1 / (1-2)	0.6 / (.4-.9)	***<0.001***
Laterality	*RT* *LT* *Both*	29(96.7%) 1(3.3%) 0(0%)	22(73.3%) 3(10%) 5(16.7%)	26(86.7%) 1(3.3%) 3(10%)	*0.079*
Hand free use	*No* *Yes*	30(100%) 0(0%)	30(100%) 0(0%)	30(100%) 0(0%)	-----

Table (3): Grades of full-scale IQ between phone nonusers and users in different age groups:

Variable		Low average	Average	High average	Superior	Gifted	P value
Total age group	***Non-users***	9(10%)	53(58.9%)	16(17.8%)	6(6.7%)	6(6.7%)	*0.435*
	Users	10(11.1%)	54(60%)	17(18.9%)	8(8.9%)	1(1.1%)	
13-18 age group	***Non-users***	2(6.7%)	17(56.7%)	8(26.7%)	1(3.3%)	2(6.7%)	***0.012***
	Users	7(23.3%)	18(60%)	1(3.3%)	4(13.3%)	-------	
6-13 age group	***Non-users***	5(16.7%)	18(60%)	4(13.3%)	3(10%)	------	*0.409*
	Users	2(6.7%)	20(66.7%)	7(23.3%)	1(3.3%)	-------	
4-6 age group	***Non-users***	2(6.7%)	18(60%)	4(13.3%)	2(6.7%)	4(13.3%)	*0.369*
	Users	1(3.3%)	16(53.3%)	9(30%)	3(10%)	1(3.3%)	

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Table (4) Comparison between mobile phone nonusers and users in the different age groups regarding serum transthyretin levels:

Age group	Serum transthyretin level		P value
	Non- users	Users	
Total age group	21.5 / (18-27.5)	28.3 / (21.4-33.3)	***<0.001***
13-18 years	25.3 / (21.9-28.5)	35.5 / (31.8-40.9)	***<0.001***
6-13 years	25.5 / (18-31)	29 / (26-31)	***0.046***
4-6 years	18 / (16.5-21)	19.8 / (16.8-22)	*0.320*

Table (5) Logistic regression analysis of transthyeretin and Full- scale IQ predicting the mobile use in children

	OR	95% CI	P value
Serum TTR in total age group	1.062	1.02-1.11	***0.003***
Serum TTR in 13-18 age subgroup	1.31	1.12-1.49	***<0.001***
Serum TTR in 6-13 age subgroup	1.021	0.097-1.08	0.441
Full scale IQ in total age group	1.01	0.9-1.03	*0.3*
Full-scale IQ in 13-18 age group	1.04	1-1.09	***0.05***
Full-scale IQ in 6-13 age group	0.8	0.60-1.1	*0.3*
Full-scale IQ in 4-6 age group	1.7	0.61-5.04	*0.2*

Table (6) Correlation between serum transthyretin levels, radiofrequency exposure, and Full-scale IQ in different age groups

Age group	Serum Trans-thyeretin							
	Total age group		13-18 age group		6-13 age group		4-6 age group	
	r	*P value*	*r*	*P value*	*r*	*P value*	*r*	*P value*
Duration of call by minutes	***0.403***	***<0.001***	***0.384***	***0.036****	*-0.149*	*0.432*	*-0.166*	*0.381*
Number of call/ weeks	***0.557***	***<0.001***	*0.263*	*0.160*	*-0.221*	*0.240*	*-0.038*	*0.842*
Duration of use / years	***0.778***	***<0.001***	***0.798***	***<0.001***	*-0.108*	*0.569*	*0.296*	*0.112*
Full-scale IQ in Users	*-0.151*	*0.155*	*0.227*	*0.229*	*0.101*	*0.594*	*0.142*	*0.454*
Full-scale IQ in Non-users	*0.074*	*0.489*	*-0.145*	*0.445*	*0.301*	*0.106*	*0.021*	*0.915*

- ***Pearson's correlation coefficient***
- ***Significant correlation at P value < 0.05***

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