



Knowledge, Attitude, and Performance of Pregnant Women Regarding General Anesthesia Neurotoxicity in Children under Three: A Report from an Academic Hospital

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Abstract

Background: Recently, concerns about general anesthesia (GA)-related neurotoxicity has been growing in societies. Parents' information obviously plays an important role to make right decision for elective surgeries on children aged under three years old.

Objectives: The aim of this survey was to evaluate the knowledge, attitude, and performance of pregnant women about the GA-related neurotoxicity in children aged under three years old.

Methods: This descriptive study was conducted at Alzahra Teaching Hospital in Guilan, Iran, during 2020. The eligible pregnant women admitted to this center were interviewed, and a questionnaire containing 10 items was filled out by the responsible resident of anesthesiology.

Results: In this research, a total of 361 pregnant women were enrolled and underwent a face-to-face interview. The mean age of the participants was 31.4 ± 7 years, 64.5% were living in urban areas, 82.5% were housewives, and 65.7% were multipara. Moreover, 83.7% of participants believed that receiving information in this regard was crucial, and 81.7% preferred physicians as the source of information. Only 8% of mothers had received information regarding the issue. A significant correlation was observed between the habitat, employment, the level of education, knowledge, and attitude status, and the source of receiving information.

Conclusions: According to our results, the knowledge, attitude, and performance of pregnant women were not optimal and needed to be improved through practical strategies.

Keywords: General Anesthesia Neurotoxicity, Knowledge, Attitude, Performance, Pregnant Women

1. Background

Recently, the topic of general anesthesia (GA)-related neurotoxicity has been growing as a big concern (1, 2). Based on experimental studies, the issue has been highly confirmed (3, 4). However, the results of human research have shown inconsistent findings. While several studies have strongly supported the potential risk of GA agents before age three, some others have reported no association between GA exposure in young children and late neurodevelopmental disorders (5-9). Since 2017, the Food and Drug Administration (FDA) has definitely recommended that elective surgeries or any procedure requiring sedation or anesthesia, which could be postponed until age above three, should not be performed in early childhood.

This risk is especially raised during repeated or prolonged anesthesia (10, 11). Indeed, GA exposure in young children should be restricted to emergency operations or vital diagnostic procedures. Therefore, awareness and attitude of the society towards the issue should be corrected, and attempt should be made to induce the concern about the possibility of GA-related risk of neurotoxicity (12, 13). Obviously, it should be noted that depriving a child of analgesia and anesthesia due to the fear of neurotoxicity is not ethically and legally acceptable. Studies have confirmed that experience of pain and severe anxiety would be very harmful with long-lasting adverse effects. Hence, they strongly recommended to use effective pain control strategies (14).

Furthermore, a balanced anesthesia could provide fa-

avorable conditions for both the child and surgery team. Pediatric anesthesia and sedation is provided via two main receptors, including Gamma aminobutyric acid (GABA) and N-methyl-D-aspartate (NMDA) (15-17). In this regard, few studies have been conducted advising an effective communication between anesthesiologists and other fields to prevent unnecessary surgeries such as circumcision before the age of three; however, few studies have focused on the parents' knowledge and attitude. In this study, for the first time, we evaluated mothers' knowledge, attitude, and performance about the GA-related neurotoxicity as a major fundamental step. Previous studies have demonstrated the mothers' desire toward being aware of the risk of anesthesia (13, 18). Pregnant women candidate for delivery will be in the decision-making position for the child's possible elective surgeries in the near future. Therefore, improving their attitude and knowledge towards the issue could be a fundamental step.

2. Objectives

The aim of this survey was to evaluate the knowledge, attitude, and performance of pregnant women about the GA-related neurotoxicity in children aged under three years old.

3. Methods

After approval of the Research Ethics Committee of Guilan University of Medical Sciences (GUMS), this descriptive study was conducted at Alzahra hospital, an academic and referral center in Guilan, Iran, during 2020.

Inclusion criteria: Term parturient women referred to Alzahra hospital for delivery; caesarean section (CS) or normal vaginal delivery (NVD) who could properly communicate.

Exclusion criteria: Women who could not communicate for any reason; those who did not give informed consent.

After the initial screening, the eligible cases were interviewed. A researcher-made questionnaire containing 10 items about knowledge, attitude and performance of mothers towards GA related neurotoxicity and long-term behavioral and neurocognitive disorders was filled out by the resident of anesthesiology. The reliability and validity of the questionnaire were confirmed by 10 faculty members. A total of 385 pregnant women were interviewed, and 24 individuals were excluded due to language differences and difficulty in communication.

3.1. Statistical Analysis

The achieved data were analyzed by SPSS software version 21. In order to find any association between variables and mothers' answers, one-way analysis of variance (ANOVA) was used. Chi-square and Fisher's exact tests were also used. A P-value less than 0.05 was considered as significant.

4. Results

A total of 361 pregnant women were interviewed. Basic demographic data of our cases are shown in Table 1. The mean age of participants was 31.4 ± 7.13 years (age range: 16 - 48 years). Also, 64.5% of participants were living in urban areas, 82.5% were housewives, 17.5% were multipara, and 2.8% were illiterate. In terms of knowledge items, most of them chose the answer "I don't know". However, 71.5% of mothers believed that GA agents during pregnancy could be harmful to the fetus, and 72.3% of them had no idea about the safe age for GA exposure. In terms of attitude items, 85.9% of them had not received any information regarding the potential risks of GA-related neurotoxicity in childhood. Moreover, 83.7% declared that receiving information regarding the issue was necessary and 81.7% preferred physicians for this purpose (Table 2). A significant correlation was observed between the residence place, occupation, and the level of education, knowledge, and attitude status, and the source of receiving information ($P \leq 0.05$). Mothers with higher level of education, living in urban areas, and having a job had better information, and physicians were the main source of their information (Tables 3 and 4).

5. Discussion

Based on the results of this study, enough attention has not been paid to the issue. Indeed, these alarming results call for a practical intervention to improve parents' knowledge and attitude towards the issue. It is undeniable that parents are involved in the child's treatment decisions. Educated mothers, who made up a small percentage of the total study population, gave acceptable answers, however in general, the participants knowledge and awareness were far from optimal status. The majority of mothers had received no information about the topic and believed that it was a critical duty of physicians. The most frequent answer regarding knowledge area was "I don't know". Indeed, they did not consider a safe age to receive anesthesia for children. However, they were aware of the fact that GA drugs could be harmful to the fetus during pregnancy, but they were not sure about its harmfulness

Table 1. Mothers' Demographic Characteristics

Variables/Status2	Number3	Percent4
Maternal age		
≤ 20	18	5
21 - 30	152	42.1
31 - 40	149	41.3
41 - 50	42	11.6
Mean ± SD (min - max)	31.4 ± 7.13 (16 - 48)	
Place of residence		
Urban	233	64.5
Rural	128	35.5
Employment Status		
Housewife	298	82.5
Employed	63	17.5
Education		
Illiterate	10	2.8
Under diploma	114	31.6
Diploma	162	44.9
University degree	75	20.8
Parity		
Primipara	124	34.3
Multipara	237	65.7

Table 2. Frequency of Mothers' Answers about Knowledge, Attitude, and Practice of GA-Related Neurotoxicity

Questions	Yes; No. (%)	No; No. (%)	I Don't Know; No. (%)
Could general anesthesia before age three be harmful for learning ability?	92 (25.5)	46 (12.7)	223 (61.8)
Could general anesthesia before age three be harmful for behavioral problems like attention deficit hyperactivity disorder (ADHD)?	91 (25.2)	52 (14.4)	218 (60.4)
Could GA agents be harmful to the fetus during pregnancy?	258 (71.5)	71 (19.7)	32 (8.9)
Is it necessary to inform parents about the potential risk of GA-related neurotoxicity?	302 (83.7)	40 (11.1)	19 (5.3)
If anesthesiologists or surgeons recommend you to postpone an elective surgery to above three, would you accept it?	93 (25.8)	243 (67.3)	25 (6.9)
Do you prefer surgery or painful diagnostic procedures without anesthesia due to the fear of anesthesia?	87 (24.1)	205 (56.8)	69 (19.1)

for their young children. In spite of the poor knowledge, they declared that if anesthesiologists or surgeons recommend to postpone an elective (and not vital surgery) to age above three, they would accept it. It is a valuable finding that indicates the important role of physicians to provide information to the parents as they obey them well and only need a reliable guidance. As expected, a significant association was observed between the place of residence, occupation, and level of education, knowledge and attitude status, and the source of receiving information. Mothers with a higher level of education, living in urban areas, and having a job had better information, and physicians were the main source of their information. Recently, the risk of anesthesia and parents' requests has been discussed as a notable topic. In line with this study, Lithman et al. evaluated the parents' knowledge and attitude regarding GA-related risk. They found that most of the par-

ents, especially mothers, were interested in receiving information regarding potential risks of anesthesia (19). Wiselo et al. also reported that parents were curious about GA in their children, and 41% of them asked for a videotape as a part of providing more information (18). In a research conducted by Nemer gut et al., it was emphasized that the potential risks of anesthetic agents on children's neuro-development should be discussed with parents, and it could be one of the items of the consent process (13). Franck et al. planned a review and analyzed the published literature on providing information to parents about pediatric anesthesia. They reported that parents preferred to receive information about pre-surgical anesthesia assessment clinic visits, anesthesia methods, potential risks, and personnel roles via different tools such as verbal, video, or written modalities. Moreover, they showed less anxiety and more satisfaction (20). There is limited litera-

Table 3. Frequency of Mothers' Answers about GA before Three and Learning Problems ^a

Could General Anesthesia Before Age Three be Harmful for Learning Ability?				
Variables	Yes	No	I Don't Know	P-Value
Maternal age; Mean \pm SD	30.51 \pm 7.25	31.71 \pm 5.71	31.71 \pm 7.34	0.377
Place of residence				0.021
Urban	66 (28.3)	22 (9.4)	145 (62.2)	
Rural	26 (20.3)	24 (18.8)	78 (60.9)	
Occupation				0.104
Housewife	73 (24.5)	34 (11.4)	191 (64.1)	
Employed	19 (30.2)	12 (19)	32 (50.8)	
Level of education				0.012
Illiterate	2 (20)	0 (0)	8 (80)	
Under diploma	19 (16.7)	20 (17.5)	75 (65.8)	
Diploma	41 (25.3)	19 (11.7)	102 (63)	
University	30 (40)	7 (9.3)	38 (50.7)	
Parity				0.605
Primipara	31 (25)	13 (10.5)	80 (64.5)	
Multipara	61 (25.7)	33 (13.9)	143 (60.3)	

^a Values are expressed as No. (%) unless otherwise indicated.**Table 4.** Frequency of Mothers' Answers about GA before Three and Behavioral Problems ^a

Could General Anesthesia Before Age Three be Harmful for Behavioral Problems Like ADHD?				
Variables	Yes	No	I Don't Know	P-Value
Maternal age; Mean \pm SD	31.18 \pm 6.02	32.59 \pm 7.27	31.22 \pm 7.52	0.433
Place of residence				0.165
Urban	62 (26.6)	28 (12)	143 (61.4)	
Rural	29 (22.7)	24 (18.8)	75 (58.6)	
Occupation				0.015
Housewife	70 (23.5)	38 (12.8)	190 (63.8)	
Employed	21 (33.3)	14 (22.2)	28 (44.4)	
Level of education				0.002
Illiterate	2 (20)	0 (0)	8 (80)	
Under diploma	25 (21.9)	20 (17.5)	69 (60.5)	
Diploma	32 (19.8)	20 (12.3)	110 (67.9)	
University degree	32 (42.7)	12 (16)	31 (41.3)	
Parity				0.02
Primipara	25 (20.2)	12 (9.7)	87 (70.2)	
Multipara	66 (27.8)	40 (16.9)	131 (55.3)	

^a Values are expressed as No. (%) unless otherwise indicated.

ture about GA-related neurotoxicity in Iran, and no similar study has been performed to investigate mothers' attitudes regarding the issue yet. Obviously, this emphasizes the novelty of the study. However, this limits the possibility of a challenging discussion and comparing the results of other studies. In a survey from northern Iran, the incidence and some related factors of elective pediatric surgeries were evaluated. It was reported that urban boys undergoing circumcision were the most cases of elective surgeries before the age of three (21). In another study, knowledge, and practice of physicians at Guilan academic hospitals regarding GA-related neurotoxicity was evaluated, and it was revealed that the current curriculum should be revised (22). In summary, it is clear that anesthesiologists' knowledge and interventions are not adequate to solve the problem, because they only visit a limited number of pregnant women who are candidate for elective cesarean delivery and not those who are in labor with NVD planning or those who undergo an emergency CS. Accordingly, anesthesiologists should have a proper communication with pediatricians, obstetrics, and surgeons in this regard. Also, obstetrics could play an important role because they frequently visit women during pregnancy and for prenatal care. Hence, they can easily communicate with them and provide the needed information.

5.1. Limitations

This study was a single-center study, and pregnant women admitted to private hospitals were not enrolled. Cultural differences, level of education, and economic status could affect the results.

5.2. Conclusion

This study revealed that mothers' knowledge, attitude, and performance are far from optimal status and require to be improved via effective and practical interventions.

Footnotes

Authors' Contribution: Study concept and design, G.B and B.N.N; Acquisition of data and drafting of the manuscript, M.M.M and Z.M; Statistical analysis, S.S; Analysis and interpretation of data, Z.R.S; Critical revision of the manuscript for important intellectual content, A.MJ and V.I.

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References

- Bong CL, Allen JC, Kim JT. The effects of exposure to general anesthesia in infancy on academic performance at age 12. *Anesth Analg*. 2013;**117**(6):1419–28. doi: [10.1213/ANE.0b013e318299a7c2](https://doi.org/10.1213/ANE.0b013e318299a7c2). [PubMed: [24132012](https://pubmed.ncbi.nlm.nih.gov/24132012/)].
- Bjur KA, Payne ET, Nemergut ME, Hu D, Flick RP. Anesthetic-related neurotoxicity and neuroimaging in children: A call for conversation. *J Child Neurol*. 2017;**32**(6):594–602. doi: [10.1177/0883073817691696](https://doi.org/10.1177/0883073817691696). [PubMed: [28424007](https://pubmed.ncbi.nlm.nih.gov/28424007/)]. [PubMed Central: [PMC5407309](https://pubmed.ncbi.nlm.nih.gov/PMC5407309/)].
- Walters JL, Chelonis JJ, Fogle CM, Ferguson SA, Sarkar S, Paule MG, et al. Acetyl-L-carnitine does not prevent neurodegeneration in a rodent model of prolonged neonatal anesthesia. *Neurotoxicol Teratol*. 2020;**80**:106891. doi: [10.1016/j.ntt.2020.106891](https://doi.org/10.1016/j.ntt.2020.106891). [PubMed: [32376384](https://pubmed.ncbi.nlm.nih.gov/32376384/)].
- Woodward TJ, Timic Stamenic T, Todorovic SM. Neonatal general anesthesia causes lasting alterations in excitatory and inhibitory synaptic transmission in the ventrobasal thalamus of adolescent female rats. *Neurobiol Dis*. 2019;**127**:472–81. doi: [10.1016/j.nbd.2019.01.016](https://doi.org/10.1016/j.nbd.2019.01.016). [PubMed: [30825640](https://pubmed.ncbi.nlm.nih.gov/30825640/)]. [PubMed Central: [PMC6588498](https://pubmed.ncbi.nlm.nih.gov/PMC6588498/)].
- Clausen NG, Kahler S, Hansen TG. Systematic review of the neurocognitive outcomes used in studies of paediatric anaesthesia neurotoxicity. *Br J Anaesth*. 2018;**120**(6):1255–73. doi: [10.1016/j.bja.2017.11.107](https://doi.org/10.1016/j.bja.2017.11.107). [PubMed: [29793593](https://pubmed.ncbi.nlm.nih.gov/29793593/)].
- Gleich SJ, Flick R, Hu D, Zaccariello MJ, Colligan RC, Katusic SK, et al. Neurodevelopment of children exposed to anesthesia: design of the Mayo Anesthesia Safety in Kids (MASK) study. *Contemp Clin Trials*. 2015;**41**:45–54. doi: [10.1016/j.cct.2014.12.020](https://doi.org/10.1016/j.cct.2014.12.020). [PubMed: [25554440](https://pubmed.ncbi.nlm.nih.gov/25554440/)]. [PubMed Central: [PMC4380751](https://pubmed.ncbi.nlm.nih.gov/PMC4380751/)].
- O'Leary JD, Janus M, Duku E, Wijeyesundera DN, To T, Li P, et al. A population-based study evaluating the association between surgery in early life and child development at primary school entry. *Anesthesiology*. 2016;**125**(2):272–9. doi: [10.1097/ALN.0000000000001200](https://doi.org/10.1097/ALN.0000000000001200). [PubMed: [27433745](https://pubmed.ncbi.nlm.nih.gov/27433745/)].
- Bellinger DC, Calderon J. Neurotoxicity of general anesthetics in children: Evidence and uncertainties. *Curr Opin Pediatr*. 2019;**31**(2):267–73. doi: [10.1097/MOP.0000000000000737](https://doi.org/10.1097/MOP.0000000000000737). [PubMed: [30720543](https://pubmed.ncbi.nlm.nih.gov/30720543/)].
- O'Leary JD. Human studies of anesthesia-related neurotoxicity in children: A narrative review of recent additions to the clinical literature. *Clin Perinatol*. 2019;**46**(4):637–45. doi: [10.1016/j.clp.2019.08.001](https://doi.org/10.1016/j.clp.2019.08.001). [PubMed: [31653299](https://pubmed.ncbi.nlm.nih.gov/31653299/)].
- US Food & Drug Administration. FDA drug safety communication: FDA review results in new warnings about using general anesthetics and sedation drugs in young children and pregnant women. USA: US Food & Drug Administration; 2017. Available from: <https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-review-results-new-warnings-about-using-general-anesthetics-and>.
- Liu X, Ji J, Zhao GQ. General anesthesia affecting on developing brain: evidence from animal to clinical research. *J Anesth*. 2020;**34**(5):765–72. doi: [10.1007/s00540-020-02812-9](https://doi.org/10.1007/s00540-020-02812-9). [PubMed: [32601887](https://pubmed.ncbi.nlm.nih.gov/32601887/)]. [PubMed Central: [PMC7511469](https://pubmed.ncbi.nlm.nih.gov/PMC7511469/)].
- Ward CG, Hines SJ, Maxwell LG, McGowan FX, Sun LS. Neurotoxicity, general anesthesia in young children, and a survey of current pediatric anesthesia practice at US teaching institutions. *Paediatr Anaesth*. 2016;**26**(1):60–5. doi: [10.1111/pan.12814](https://doi.org/10.1111/pan.12814). [PubMed: [26559907](https://pubmed.ncbi.nlm.nih.gov/26559907/)].
- Nemergut ME, Aganga D, Flick RP. Anesthetic neurotoxicity: What to tell the parents? *Paediatr Anaesth*. 2014;**24**(1):120–6. doi: [10.1111/pan.12325](https://doi.org/10.1111/pan.12325). [PubMed: [24283891](https://pubmed.ncbi.nlm.nih.gov/24283891/)].

14. Sedighie L, Bolourchifard F, Rassouli M, Zayeri F. Effect of comprehensive pain management training program on awareness and attitude of ICU nurses. *Anesth Pain Med.* 2020;**10**(2). e98679. doi: [10.5812/aapm.98679](https://doi.org/10.5812/aapm.98679). [PubMed: [32754429](https://pubmed.ncbi.nlm.nih.gov/32754429/)]. [PubMed Central: [PMC7341110](https://pubmed.ncbi.nlm.nih.gov/PMC7341110/)].
15. Omara AF, Abdelrahman AF, Elshiekh ML. Recovery with propofol anesthesia in children undergoing cleft palate repair compared with sevoflurane anesthesia. *Anesth Pain Med.* 2019;**9**(3). e92076. doi: [10.5812/aapm.92076](https://doi.org/10.5812/aapm.92076). [PubMed: [31497524](https://pubmed.ncbi.nlm.nih.gov/31497524/)]. [PubMed Central: [PMC6712429](https://pubmed.ncbi.nlm.nih.gov/PMC6712429/)].
16. Faghihian R, Eshghi A, Faghihian H, Kaviani N. Comparison of oral melatonin and midazolam as premedication in children undergoing general anesthesia for dental treatment. *Anesth Pain Med.* 2018;**8**(2). e64236. doi: [10.5812/aapm.64236](https://doi.org/10.5812/aapm.64236). [PubMed: [30009151](https://pubmed.ncbi.nlm.nih.gov/30009151/)]. [PubMed Central: [PMC6035375](https://pubmed.ncbi.nlm.nih.gov/PMC6035375/)].
17. Oriby ME. Comparison of intranasal dexmedetomidine and oral ketamine versus intranasal midazolam premedication for children undergoing dental rehabilitation. *Anesth Pain Med.* 2019;**9**(1). e85227. doi: [10.5812/aapm.85227](https://doi.org/10.5812/aapm.85227). [PubMed: [30881910](https://pubmed.ncbi.nlm.nih.gov/30881910/)]. [PubMed Central: [PMC6412317](https://pubmed.ncbi.nlm.nih.gov/PMC6412317/)].
18. Wisselo TL, Stuart C, Muris P. Providing parents with information before anaesthesia: what do they really want to know? *Paediatr Anaesth.* 2004;**14**(4):299–307. doi: [10.1046/j.1460-9592.2003.01222.x](https://doi.org/10.1046/j.1460-9592.2003.01222.x). [PubMed: [15078374](https://pubmed.ncbi.nlm.nih.gov/15078374/)].
19. Litman RS, Perkins FM, Dawson SC. Parental knowledge and attitudes toward discussing the risk of death from anesthesia. *Anesth Analg.* 1993;**77**(2):256–60. doi: [10.1213/00000539-199308000-00008](https://doi.org/10.1213/00000539-199308000-00008). [PubMed: [8346823](https://pubmed.ncbi.nlm.nih.gov/8346823/)].
20. Franck LS, Spencer C. Informing parents about anaesthesia for children's surgery: A critical literature review. *Patient Educ Couns.* 2005;**59**(2):117–25. doi: [10.1016/j.pec.2004.11.002](https://doi.org/10.1016/j.pec.2004.11.002). [PubMed: [16257615](https://pubmed.ncbi.nlm.nih.gov/16257615/)].
21. Biazar G, Farzi F, Naderi Nabi B, Atrkarroushan Z, Chaibakhsh Y, Ros-tami Lima S. General anesthesia-related neurotoxicity: Status of pediatric surgeries at an academic hospital in the north of Iran. *J Compr Pediatr.* 2019;**10**(4). doi: [10.5812/compreped.92316](https://doi.org/10.5812/compreped.92316).
22. Sedighinejad A, Soltanipour S, Rimaz S, Biazar G, Chaibakhsh Y, Badri Kouhi M. General anesthesia-related neurotoxicity in the developing brain and current knowledge and practice of physicians at Guilan academic hospitals. *Anesth Pain Med.* 2019;**9**(4). e92366. doi: [10.5812/aapm.92366](https://doi.org/10.5812/aapm.92366). [PubMed: [31750093](https://pubmed.ncbi.nlm.nih.gov/31750093/)]. [PubMed Central: [PMC6820294](https://pubmed.ncbi.nlm.nih.gov/PMC6820294/)].