Association between different parameters of child maltreatment and global DNA methylation

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ABSTRACT
Exposure to early life adversities, as child maltreatment may disturb the whole biological development of a child resulting in different consequences. Among the main underlying mechanisms are epigenetics and DNA methylation.

The aim of the study was to assess the effects of various factors such as child maltreatment (single or multiple), duration, frequency and age of onset of abuse on DNA methylation.

Subjects and methods: 90 children (55 males, 35 females) aged 12–18 years were recruited from adolescent psychiatric outpatient clinics at Al-Hadara University Hospital in Alexandria, Egypt. A Childhood Trauma Questionnaire was used to take the history of child maltreatment. Global DNA methylation was determined by MethylFlash methylated DNA quantification colorimetric kit.

Results: Regarding DNA methylation, there was a statistically significant hypomethylation was detected among those who reported exposure to more than one type of child maltreatment than those who reported no exposure or exposure to only one type. Significant difference was also detected between children who were exposed at the age of nine or less and those above 9 years. Moreover, a significant association was found between the duration and frequency of maltreatment in the prediction of DNA methylation.

Conclusion: global DNA hypomethylation can be used as a marker to detect exposure to multiple types of child maltreatment. However, it is not about the early child maltreatment alone, but the accumulation of abuse over time and higher frequency of exposure to child maltreatment that causes more global DNA hypomethylation.

1. Introduction
Child maltreatment is a complex and difficult issue to study. It includes any form of abusive behavior toward children, whether frequent or infrequent [1]. Besides being a violation of children’s human rights, abused children, compared to the non-abused, are more likely to experience a range of immune, nervous and sexually transmitted diseases [2–4].

In fact, theories by which early adversity resulting in these outcomes have been a topic of intense research. One of these theories is “epigenetics” which was first identified by Waddington C.H. in 1942 [5,6]. It refers to the external modifications of DNA that turn genes “on” or “off.” These modifications do not change the DNA sequence, instead they affect gene expression and how cells “read” genes. In addition, modification of gene expression can occur through different epigenetic changes including DNA methylation [7,8].

DNA methylation is the closest epigenetic alteration to the gene. It occurs by the addition of a methyl group onto cytosine rings in gene promoters. It also occurs in sites where cytosine nucleotide is followed by a guanine nucleotide in the 5’→3’ direction. This DNA methylation is regular, naturally occurring and essential for normal development. It results in gene silencing and suppressing its expression in promoters [9].

It is suggested that early life is a particularly sensitive period to the epigenetic mechanisms. During this “critical” period, different environmental signals would affect development [10].

The aim of the present study was to examine the impact of exposure to child maltreatment, whether one or more types, and the effect of various factors such as the duration, frequency, and age of onset of exposure to child maltreatment on DNA methylation.

2. Methods
2.1. Ethics statement
Informed consent and oral assent were obtained from caregivers and adolescents, respectively. All adolescents participated in the present study were not financially compensated for their participation. Confidentiality of all data was considered. Ethical approval was obtained from the ethical committee of Alexandria University. (IRB NO: 00012098, FWA NO: 00018699).
2.2. Study participants

A descriptive cross-sectional study was carried out on 90 adolescents (55 males and 35 females) aged 12–18 years recruited from adolescent psychiatric outpatient clinics at Al-Hadara University Hospital in Alexandria, Egypt. Children with congenital developmental anomalies, neurological diseases or malignancies were excluded from the study. Moreover, children with psychiatric disorders that lead to severe cognitive impairment and psychotic disorders were excluded. All participants were interviewed during their first visit to the psychiatric clinic before receiving any pharmacotherapy or psychotherapy. We specifically selected the adolescence period to study the DNA methylation to avoid the inter-individual epigenetic divergence with the advance of age [11]. Also, we selected the age of nine as a cut-off point depending on the neurobiology theory of early experiences [12].

For every research participant, the following was determined:

1. Demographic data (age and sex)
2. History of child maltreatment:
   - Assessment of exposure to different types of child maltreatment; emotional, physical, sexual abuse, and emotional or physical neglect, was done using the Arabic version of the short form of Childhood Trauma Questionnaire (CTQ-SF) [13].
   - The CTQ-SF is a retrospective, self-administered measure assessing each type of maltreatment by five items to provide adequate reliability and content coverage.
   - Additional information was collected to determine the age of onset of maltreatment, its duration, and frequency.
3. Epigenetics – global DNA methylation analysis:
   - Blood samples were collected from all participants in EDTA tubes and DNA extraction was performed using QIAamp blood DNA isolation kit (spin protocol) (QIAGEN) [14].
   - Global DNA methylation in blood samples of research participants was determined by measuring 5-methylcytosine (5-mC) using MethylFlash methylated DNA quantification colorimetric kit (Epigentek Group Inc) [15].
   - In this assay, DNA is bound to strip wells that are specifically treated to have a high DNA affinity. The methylated fraction of DNA is detected using capture and detection antibodies (anti-5-mC monoclonal antibodies) that is both sensitive and specific for 5-mC. The 5mC amount was quantified using a colorimeter by reading the absorbance in a microplate spectrophotometer. The amount of methylated DNA is proportional to the optic density intensity measured.

2.3. Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov – Smirnov, Shapiro and D’Agostino tests were used to verify the normality of distribution of variables. Comparisons between groups for categorical variables were assessed using Chi-square test (Monte Carlo). Student t-test was used to compare two groups for normally distributed quantitative variables, while ANOVA was used for comparing the four studied groups. Pearson coefficient was applied to correlate between two normally distributed quantitative variables. Regression analysis was done to detect the most independent affecting factor for DNA methylation. Significance of the obtained results was judged at the 5% level.

3. Results

1. Demographic data and history of child maltreatment:
   - Less than half (47.8%) of the studied adolescents were in the age group 12–14 years. This was followed by the age group 16–18 years (30%), then the age group of 14–16 years (22.2%). Regarding the sex distribution of the study participants, less than two-thirds (61.1%) were males while 38.9% were females.
   - In the current study, 73.3% of the studied participants reported exposure to different types of child maltreatment. Out of those who were exposed to child maltreatment; 42.2% reported exposure to emotional abuse, 65.6% exposed to physical abuse and 8.9% exposed to sexual abuse. Regarding neglect; emotional neglect was detected in 33.3% and physical neglect in only 1.1%. On the other hand, only 26.7% of the studied participants denied exposure to any type of child maltreatment.
   - In addition, more than one quarter (27.8%) of the study subjects were exposed to only one type of maltreatment, followed by three types of child maltreatment (23.2%) then two types (17.8%). On the other hand, only four children (4.4%) reported exposure to four types of child maltreatment (Figure 1).
   - Regarding the age of onset of child maltreatment, 71.2% of the cases reported exposure at the age of 9 years or less and 28.8% were exposed when they were more than 9-years old. The mean age of onset of exposure to child maltreatment was 7.71 ± 1.90 years.
   - About half of children (53.3%) reported exposure to child maltreatment repeatedly for a period of 1–3 years, followed by 37.9% who reported exposure for 4–6 years and 9.1% who declared...
exposure for 7–10 years. In addition, 25.8% of the study participants reported exposure to child maltreatment for more than 10 times, 15.2% reported frequency of 6–10 times, 12.1% reported frequency of 3–5 times and 9.1% reported frequency of 1–2 times only.

2. Relation between number of types of child maltreatment and different parameters:

In the current work, a significant difference was detected between those who were exposed to one, two, three and four types of child maltreatment and the age of onset of maltreatment, its duration, and frequency where \( p < 0.001, <0.001 \text{ and } 0.005 \), respectively (Table 1).

3. Level of DNA methylation in relation to different parameters:

- Age and sex:

A non-significant statistical difference was noted in the mean values of DNA methylation level between different age groups (12–14, 14–16 and 16–18 years) where \( p = 0.483 \). Moreover, it was found that there was no statistically significant difference between males and females regarding the level of DNA methylation where \( p = 0.269 \).

- Exposure to multiple types of child maltreatment:

The lowest mean level of DNA methylation was present in those children exposed to three types of child maltreatment (0.20 ± 0.02) followed by four types (0.23 ± 0.03) and then two types (0.23 ± 0.05). On the other hand, the highest mean was detected in children who were exposed to one type of child maltreatment (0.29 ± 0.04) and in those who reported no exposure to child maltreatment (0.28 ± 0.06). The difference between the five groups regarding the DNA methylation was found to be statistically significant where \( p < 0.001 \) (Table 2).

- Age of onset of child maltreatment:

In the present work, there was a statistically significant difference between study participants who reported

| Table 1. Relation between number of types of child maltreatment and different parameters (n = 66). |
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| Types of child maltreatment | One (n = 25) | Two (n = 16) | Three (n = 21) | Four (n = 4) | \( \chi^2 \) | \( p \) |
| --- | --- | --- | --- | --- | --- | --- |
| Age of onset of child maltreatment (years) | | | | | | |
| <9 | 11 44.0 | 12 75.0 | 21 100.0 | 3 75.0 | 1x.381* | MC \( p < 0.001^* \) |
| \( \geq 9 \) | 14 56.0 | 4 25.0 | 0 0.0 | 1 25.0 | | |
| Duration of exposure to child maltreatment (years) | | | | | | |
| 1–3 | 23 92.0 | 5 31.3 | 5 23.8 | 2 50.0 | 29.373 | <0.001* |
| 4–6 | 2 8.0 | 7 43.8 | 14 66.7 | 2 50.0 | | |
| 7–10 | 0 0.0 | 4 25.0 | 2 9.5 | 0 0.0 | | |
| Frequency of exposure to child maltreatment | | | | | | |
| 1–2 | 6 24.0 | 0 0.0 | 0 0.0 | 0 0.0 | 23.853 | 0.005* |
| 3–5 | 6 24.0 | 2 12.5 | 0 0.0 | 0 0.0 | | |
| 6–10 | 4 16.0 | 2 12.5 | 3 14.3 | 1 25.0 | | |
| \( >10 \) | 1 4.0 | 7 43.8 | 8 38.1 | 1 25.0 | | |
| Cannot remember | 8 32.0 | 5 31.3 | 10 47.6 | 2 50.0 | | |

\( \chi^2 \): Chi square test.

\( p \): p value for association between types of child maltreatment and different parameters.

\( ^* \): Statistically significant at \( p \leq 0.05 \).
exposure to child maltreatment at the age of nine or less and those who were exposed above the age of nine where \( p = 0.001 \) (Table 2).

- **Duration of exposure to child maltreatment:**

  The mean of DNA methylation was the highest (0.27 ± 0.05) in those children who were exposed to child maltreatment for the shortest duration (1–3 years). A statistically significant difference was present between means of DNA methylation of cases with different durations of exposure to child maltreatment where \( p < 0.001 \) (Table 2).

- **Frequency of exposure to child maltreatment:**

  Moreover, the mean of DNA methylation was the highest (0.31 ± 0.02) in those who were exposed in a frequency of 1–2 times followed in a descending manner by those who were exposed in a frequency of 3–5 times (0.29 ± 0.03) followed by those exposed in a frequency of 6–10 times (0.24 ± 0.05). In addition, the lowest mean of DNA methylation was detected in those children exposed to a frequency more than 10 times (0.21 ± 0.04). A statistically significant difference was detected between the means of DNA methylation of cases with different frequency of exposure to child maltreatment (Table 2).

  In addition, multivariate linear regression was done in the current work to examine the effect of each type of child maltreatment on the level of DNA methylation. Significant correlation was detected between physical abuse and the level of DNA methylation. A significant correlation was noted between the duration and frequency of exposure to child maltreatment and level of DNA methylation (Table 4).

4. **Discussion**

Biological development passes through a sensitive period starting with the prenatal period and reaches its peak in the first few years in life. So, exposure to early life adversity, as child maltreatment, would disturb the whole biological development of a child resulting in different consequences. Among the underlying mechanisms, it is thought that epigenetic processes are involved [16].

In the present study, we were concerned with the impact of child maltreatment, exposure to one or more than one type, exposure’s duration and frequency as well as age of onset of exposure on DNA methylation.

It was found that nearly three-quarters of the studied participants were exposed to child maltreatment. The highest percentage reported physical, emotional abuse, and emotional neglect. However, sexual abuse and physical neglect were only present at 8.9% and 1.1%, respectively. This is largely consistent with a survey performed by UNICEF in Alexandria, Egypt, which reported that 65% and 72% of children between 13 and 17 years reported exposure to physical

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**Table 2. Relation between DNA methylation and different parameters of the studied child maltreatment cases.**

| Child maltreatment             | N  | Min. – Max. | Mean ± SD | Median | Test of sig. | p    |
|--------------------------------|----|-------------|-----------|--------|--------------|------|
| Non exposed                    | 24 | 0.17–0.37   | 0.28 ± 0.06 | 0.29   | F = 14.266*  | <0.001*|
| Exposed to one type            | 25 | 0.16–0.34   | 0.29 ± 0.04 | 0.30   |              |      |
| Exposed to two types           | 16 | 0.16–0.30   | 0.23 ± 0.05 | 0.22   |              |      |
| Exposed to three types         | 21 | 0.16–0.25   | 0.20 ± 0.02 | 0.20   |              |      |
| Exposed to four types          | 4  | 0.21–0.27   | 0.23 ± 0.03 | 0.21   |              |      |
| Age of onset child maltreatment (years) (n = 66) |    |             |           |        |              |      |
| <9                             | 47 | 0.16–0.34   | 0.23 ± 0.05 | 0.21   | t = 3.611*   | 0.001*|
| ≥9                            | 19 | 0.16–0.33   | 0.28 ± 0.05 | 0.29   |              |      |
| Duration of exposure to child maltreatment (years) (n = 66) |    |             |           |        |              |      |
| 1 – 3                          | 35 | 0.16–0.34   | 0.27 ± 0.05 | 0.29   | F = 14.744*  | <0.001*|
| 4 – 6                          | 25 | 0.16–0.30   | 0.21 ± 0.04 | 0.19   |              |      |
| 7 – 10                         | 6  | 0.16–0.29   | 0.22 ± 0.05 | 0.22   |              |      |
| Frequency of exposure to child maltreatment (n = 66) |    |             |           |        |              |      |
| 1 – 2                          | 6  | 0.28–0.33   | 0.31 ± 0.02 | 0.32   | F = 8.836*   | <0.001*|
| 3 – 5                          | 8  | 0.24–0.33   | 0.29 ± 0.03 | 0.30   |              |      |
| 6 – 10                         | 10 | 0.16–0.31   | 0.24 ± 0.05 | 0.24   |              |      |
| >10                            | 17 | 0.16–0.29   | 0.21 ± 0.04 | 0.20   |              |      |
| Cannot remember                | 25 | 0.16–0.34   | 0.23 ± 0.05 | 0.21   |              |      |

F: F for ANOVA test t: Student t-test.

\( p \): p value for association between DNA methylation and different parameters.

\*: Statistically significant at \( p \leq 0.05 \).
The highest mean was present in those children who were exposed to physical neglect. A statistically significant difference was detected in children who were exposed to one type of child maltreatment followed by those who reported no exposure to child maltreatment. This finding was unexpected, but it could be explained by the dynamic changing nature of epigenetics even in those with the lowest level, alterations, and positive outcome is still possible [19].

Moreover, this reflects the presence of other variables influencing this epigenetic mechanism and resulting in this dysregulation. Among these variables is diet. In fact, DNA methylation depends on dietary methionine and folate. So, eating food poor in methionine has been reported to lead to DNA hypomethylation [4].

In addition, this finding is supported by Bodden et al. [20] study who thought that some adversity may be beneficial. Moreover, Bateson et al. [21] suggested that cues received in early life would influence the development of a phenotype resulting in normal adaptation to later environmental conditions.

Also, this difference would be attributed to the significant difference between number of maltreatment types and age of onset, duration, and frequency of maltreatment exposure. For example, in the current work, more than half of those who exposed to only one type and has the highest DNA methylation started exposure at age above nine, more than 90% of them exposed for 3 years or even less and only 4% of them reported a long exposure frequency more than 10 times.

So, we tried to examine the relation between DNA methylation and the age of onset of the child maltreatment. Although there is a wide variation between different literatures in describing the word “early age”, the age of nine was used as a cutoff point in the current work [22,23]. It was based on the neurobiology theory of early experiences. This theory is based on the prefrontal cortex, which controls the response to different social and emotional stimuli and its development from the age of three to 9 years. So according to this theory, children who are exposed to child maltreatment early before prefrontal cortex development will be affected more than other children [12].

| Frequency of exposure to child maltreatment (years) | B     | SE    | Beta  | t     | p    |
|---------------------------------------------------|-------|-------|-------|-------|------|
| Age of onset child maltreatment (years)            | -0.012| 0.004 | -0.301| 2.802 | 0.007*|
| Duration of exposure to child maltreatment (years) | -0.009| 0.004 | -0.348| 2.362 | 0.022*|
| Frequency of exposure to child maltreatment        | -0.006| 0.004 | -0.201| 1.496 | 0.140|
| Physical neglect (years)                          | -0.002| 0.001 | -0.170| 1.497 | 0.140|
| Emotional neglect (years)                         | -0.002| 0.001 | -0.162| 1.644 | 0.106|
| Sexual abuse (years)                               | -0.003| 0.001 | -0.167| 1.148 | 0.256|
| Physical abuse (years)                             | -0.001| 0.001 | -0.144| 1.203 | 0.234|
| Emotional abuse (years)                            | -0.001| 0.001 | -0.170| 1.497 | 0.140|
| Physical neglect (years)                           | -0.002| 0.001 | -0.162| 1.644 | 0.106|
| Emotional neglect (years)                          | -0.002| 0.001 | -0.167| 1.148 | 0.256|
| Sexual abuse (years)                               | -0.003| 0.001 | -0.170| 1.497 | 0.140|
| Physical abuse (years)                             | -0.001| 0.001 | -0.144| 1.203 | 0.234|
| Emotional abuse (years)                            | -0.001| 0.001 | -0.170| 1.497 | 0.140|
Results of the current study coincide with this theory; DNA methylation was significantly lower in children who were exposed to child maltreatment at the age of nine or less than those who started to be exposed above 9 years.

In addition, the current study revealed a statistically significant difference between the DNA methylation and the duration of exposure to child maltreatment, where the highest DNA methylation mean was recorded in those who were exposed to the shortest duration of child maltreatment. On the other hand, those children who were exposed to the longest duration of maltreatment (7–10 years) had a higher methylation than those who were exposed for 3–6 years duration. In our opinion, this reflects the reversibility of the negative impact of toxic stress [19]. It also reflects the presence of other factors affecting DNA methylation such as the severity of abuse itself.

Moreover, the current study showed a statistically significant difference between DNA methylation and the frequency of exposure to child maltreatment. The highest mean was detected in those exposed to the least frequency of maltreatment. However, there is no reliable method of measuring the frequency of child maltreatment. Even in the current study more than one-third of children didn’t remember the frequency of their exposure.

In addition, multivariate linear regression was done in the current work to examine the effect of each type of child maltreatment on the level of DNA methylation. Significant correlation was detected between physical abuse and the level of DNA methylation. Many literatures discussed the effect of physical abuse on DNA methylation as Yang et al. [24], Martin-Blanco et al. [25] and Radtk et al. [16]. Moreover, Suderman et al. [26] studied the DNA promoter methylation from 20,533 genes associated with bullying, emotional and verbal abuse and found differential methylation; 686 hypomethylated regions versus 311 hypermethylated regions. DNA hypomethylation is associated with genomic instability and chromosomal aberration. This would give a good explanation of the long-term consequences of early life adversity and the mediating role of epigenetics [27].

On the other hand, a non-significant correlation was detected between exposure to sexual abuse and the level of DNA methylation. This finding throws the light on a meta-analysis based on community and college samples which suggested that child sexual abuse is weakly associated with later problems [28,29].

However, these results were in contrast to Beach et al. [30] and Suderman et al. [26] studies, who established an association between sexual abuse and epigenetic changes. This difference could be attributed to the small number of cases of sexual abuse included in the present study.

In our opinion, children who were victims of sexual abuse are considered a vulnerable group and received a good support from their family and community. This could explain the absence of this adversity effect on DNA methylation. Unfortunately, other maltreatment types, especially physical abuse, are an acceptable method of discipline in our culture and is supported. So, those children don’t receive any support instead are subjected to more and more blame.

Furthermore, to judge child maltreatment-epigenetic correlation in the present work, multivariate linear regression was done to examine the effect of age of onset, duration, and frequency of child maltreatment and its interaction with different forms of child maltreatment on the level of DNA methylation. A significant correlation was found between the duration and frequency of exposure to child maltreatment in the prediction of DNA methylation. This is a very important finding as it is not only about the early life adversity but it is the accumulation over a lifetime for a longer duration and higher frequency of exposure that causes more global DNA hypomethylation. The longer the duration and the repeated the exposure, the less time there is to reverse the process.

In the past, prevention of child maltreatment was the only way to stop its long-term consequences. However, studying the association between child maltreatment and epigenetic changes and the mediating role of epigenetics in causing the long-term consequences, besides the reversible nature of epigenetics, would open a hopeful door of cure for all maltreatment victims.

5. Conclusion

- There is a significant correlation between exposure to physical abuse and level of global DNA methylation.
- The lowest mean level of DNA methylation was present in those children exposed to more than one type of child maltreatment than those who declared exposure to only one type.
- DNA methylation was significantly lower among those who had experienced child maltreatment at a younger age, for the longest duration, and in high frequencies.
- A significant correlation was found between the duration and frequency of exposure to child maltreatment in the prediction of DNA methylation.

6. Recommendations

Further studies are needed to follow up global DNA hypomethylation, and other factors affecting its reversibility among child maltreatment cases. In addition, other analytical studies are needed to compare the effect of exposure to one type versus exposure to
multiple child maltreatment on DNA methylation while controlling other confounding variables.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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