Research Article

Factor V Leiden Mutation in Egyptian Children with Cerebral Palsy

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Abstract

Objective: Gene mutations are known to play a role in the development of cerebral palsy (CP). The aim of this study was to determine the frequency of Factor V Leiden (FVL) mutation in Egyptian CP Children. **Patients and Methods:** The study included ٧٠ children; ٥٠ patients with CP (Group I) and ٢٠ healthy subjects (Group II) matched age and sex-were chosen as control group. Venous blood samples were used for DNA extraction using PCR testing. PCR primers were designed based on Exon ١٠ sequence of human factor v gene.
Results: There was statistically non significant difference between both groups regarding comparison of demographic characteristics and risk factors except fro pre-term birth (٢٦٪ in study group versus ٥٪ in control group, P= ٠.٠٤). The frequency of fVL mutation was ٤٢٪ in the study group, ١٥٪ in control group, and ٤٣.٤٪ for all studied participants, with a statistically significant difference between study and control groups. There was a significant association between homozygous fVL mutation and severe type of CP; ٦٠٪ of homozygous mutations associated with severe CP versus ٩٪ of heterozygous mutations. **Conclusion:** The fVL mutation is one of a number of potential factors that may increase the likelihood of cerebral thrombo-embolism and subsequent CP in Egyptian children.
Keywords: Cerebral palsy, risk factors, factor V Leiden, mutation.

Introduction

Cerebral palsy (CP) is a heterogeneous condition with multiple causes; multiple clinical types; multiple patterns of neuro-pathology on brain imaging; multiple associated developmental pathologies, such as intellectual disability, autism, epilepsy, and visual impairment; and more recently multiple rare pathogenic genetic mutations [١].

Globally, CP is a common neurologic problem in children and is reported as occurring in approximately ١.٥-٣ of ١٠٠٠ live births[٢,٣]. In Egypt, the prevalence of CP in children was ٣.٦ per ١,٠٠٠ live-birth in another study recorded in Al-Quseir City. The currently high prevalence of CP in our country may be attributed to an improved survival rate of preterm and low birth weight infants reported with CP[٤].

While CP was initially attributed to injuries resulting from birth asphyxia, recent studies have shown that in actuality it includes a myriad of factors. Injury to the developing

brain may be prenatal, natal or postnatal. Risk factors now known to play a role in the development of CP include multiple gestation, gender, infection, prematurity and low birth weight as well as genetic determinants[٥]. Mutations in genes associated with the coagulation cascade trigger hypercoagulable states (hereditary thrombophilia) that, in theory, increase the risk of CP[٦]. The factor V Leiden (FVL) mutation is the most common form of hereditary thrombophilia, and heterozygosity increases the risk of thrombosis three- to sevenfold[٧].

The aim of this study was to determine the frequency of Factor FVL mutation in Egyptian CP Children In Minia

Governorate, and to ascertain whether children with CP have higher frequency of factor V leiden mutation compared with normal children, aiming to decrease the frequency of occurrence of CP by diagnosing the mutation and trying to control the environmental circumstances.

Patients and Methods

The study included ٧٠ children; ٥٠ patients with CP (Group I; study group) selected from the out-patient pediatric neurology clinic and pediatric in-patients department in Minia University Children Hospital, in the period from November ٢٠١٤ to July ٢٠١٥. In addition to ٢٠ healthy subjects (Group II; control group) matched age and sex-were chosen as control group. Children with cerebral palsy secondary to CNS infections, Kernicterus, head trauma or intracranial hemorrhage were excluded.

All patients and their families were interviewed in details of thorough history (demographic characteristics, consanguinity, pregnancy, delivery, perinatal events, etc.). All participtants were subjected to complete general examination and full neurological examination, Brain CT Scan, and laboratory investigations.

Venous blood samples were collected from all participtants using standard phlebotomy. The samples were used for DNA extraction. Blood samples were centrifuged at ٣٠٠٠ rpm for ١٠ minutes plasma was separated and frozen at -٢٠°C until processed for PCR testing. PCR primers were designed based on Exon ١٠ sequence of human factor v gene. The primers used in the PCR amplification reactions include a common primer ٥'-ACTCTTAGAGTT TGATGA-٣', a normal allelspeciefic Primer ٥'-GGACCAAAATACCTGTATTCCGC-٣' and a mutation specific primer a ٥'-GGACAAAATACCTGTATTCCCT-٣'.

Statistical analyses were performed using SPSS software, version ١٨.٠ (SPSS, Chicago, IL, USA). Data were presented as mean and standard deviation (SD) for quantitative variables, or as number and percent for categorical (qualitative) variables. For univariate analysis, two-sided t-student used was used to compare independent groups of quantitative data, and Chi-square test was used to compare independent groups of qualitative data. The statistical significance of the used tests for analysis was considered when P-value was less than ٠.٠٥

Results

The study group included ٥٠ CP children with an average age of ٣.٦ ± ٢.٤ years (٢٩ male and ٢١ female), while the control group included ٢٠ children with an average age of ٤.٧±٣.٧ years (١٢ male and ٨ female). There was statistically non significant difference regarding comparison of demographic characteristics (Age and gender) and risk factors (consanginuity, maternal risk factors, Cesarean delivery) except fro pre-term birth (٢٦٪ in study group versus ٥٪ in control group, P= ٠.٠٤) between the studied groups (Table ١). The neurological motor disorders in children with CP (Figure ١) were spastic in ٤٥(٩٠٪), atonic in ٣(٦٪) and athetotic in ٢(٤٪).

The frequency of fVL mutation was ٤٢٪ in the study group, ١٥٪ in control group, and ٤٣.٤٪ for all studied participants, with a statistically significant difference between study and control groups (Figure ٢). All ٣ cases of fVL mutations in control group were heterozygous, while in the study group there were ١٠ cases of homozygous mutations and ١١ cases of heterozygous mutations, with statistically non significant difference between both groups. There was statistically non significant difference regarding comparison of demographic characteristics and risk factors between children with and without fVL mutation (Table ٢). There was a significant association between homozygous fVL mutation and severe type of CP; ٦٠٪ of homozygous mutations associated with severe CP versus ٩٪ of heterozygous mutations (Table ٣).

Table (١): Demographic characteristics and risk factors in the studied groups

Variable	Control group (n=٢٠)	Study group (n=٥٠)	P-value
Age (years)	٤.٧ ± ٣.٧	٣.٦ ± ٢.٤	٠.١٥
Male gender	١٢ (٦٠٪)	٢٩ (٥٨٪)	٠.٨٧
Consanguinity	٢ (١٠٪)	١٤ (٢٨٪)	٠.١٠
Maternal thyroid disease	٠	١ (٢٪)	٠.٥٢
Preeclampsia	١ (٥٪)	٣ (٦٪)	٠.٨٧
Maternal infection	١ (٥٪)	٤ (٨٪)	٠.٦٦
Multiple pregnancy	٠	٢ (٤٪)	٠.٣٦
Cesarean delivery	٢ (١٠٪)	٧ (١٤٪)	٠.٦٧
Pre-term birth	١ (٥٪)	١٣ (٢٦٪)	٠.٠٤*

*significant difference.

Table (٢): Demographic characteristics and risk factors in relation to presence of factor V Leiden (fVL) mutation.

Variable	fVL mutation (n=٢٤)	No fVL mutation (n=٤٦)	P-value
Age (years)	٣.٥±١.٩	٤.١± ٣.٤	٠.٣٩
Male gender	١٤ (٥٨٪)	٢٧ (٥٩٪)	٠.٩٧
Consanguinity	٧ (٢٩.٢٪)	٩ (١٩.٦٪)	٠.٣٦
Maternal thyroid disease	٠	١ (٢٪)	٠.٤٦
Preeclampsia	٢ (٨٪)	٢ (٤٪)	٠.٤٩
Maternal infection	١ (٤٪)	٤ (٩٪)	٠.٤٨
Multiple pregnancy	١ (٤٪)	١ (٢٪)	٠.٦٣
Cesarean delivery	٤ (١٧٪)	٥ (١١٪)	٠.٤٩
Pre-term birth	٥ (٢١٪)	٩ (٢٠٪)	٠.٩٠

Table (٣): Relation of the severity of cerebral palsy (CP) to the type of factor V Leiden (fVL) mutation

Severity of CP	Type of fVL mutation		P-value
	Homozygous (n=١٠)	Heterozygous (n=١١)	
Mild	٢ (٢٠٪)	٤ (٣٦٪)	٠.٤٠
Moderate	٢ (٢٠٪)	٦ (٥٥٪)	٠.١٠
Severe	٦ (٦٠٪)	١ (٩٪)	٠.٠١*

*significant difference.

Table (٤): Summary of the studies evaluated the direct association between cerebral palsy and hereditary thrombophilias.

Reference	Study type	Cases (n)	Control (n)	Analyzed genes	Results
Nelson et al., ١٩٩٨	Case-Control	٣١	٦٥	fVL	Significant increase
Fattal-Valevski et al., ٢٠٠٥	Case-Control	٤٩	١١٨	fVL, MTHFR C٦٧٧T, G٢٠٢١٠A	Non-significant difference
Yehezkely-Schildkrau et al., ٢٠٠٥	Case-Control	٦١	٦٢	fVL, PT٢٠٢١٠, MTHFR C٦٧٧ T	Non-significant difference
Reid et al., ٢٠٠٦	Case-Control	٥٧	١٦٧	fVL	Significant difference
Wu et al., ٢٠١١	Case-Control	١٣٨	١٦٥	fVL, PT٢٠٢١٠, MTHFR C٦٧٧ T	Non-significant difference
Arenas-Sordo et al., ٢٠١٢	Case-Control	٩٤	١٢٠	fVL	Non-significant difference

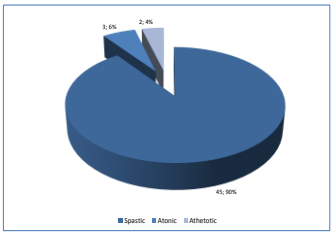

Figure (١): Distribution of neurological motor disorders in ٥٠ children with cerebral palsy**.**

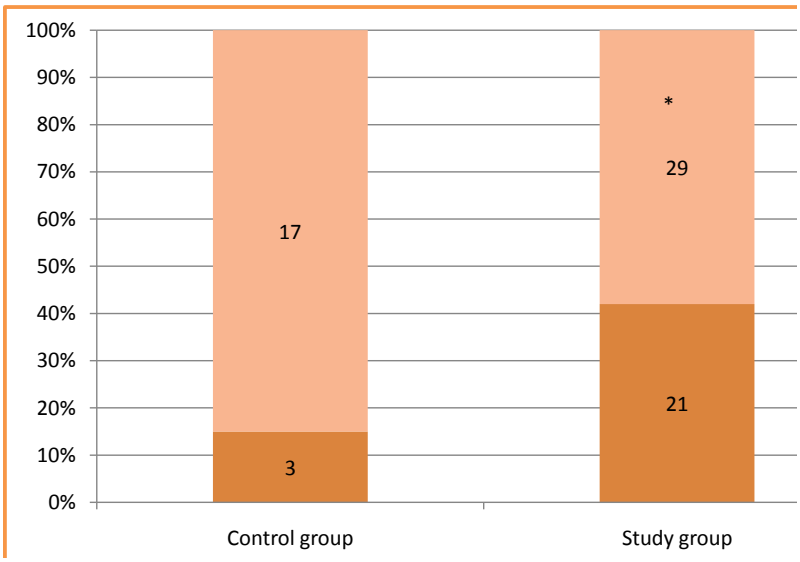

Figure (٢): Frequency of factor V Leiden (fVL) mutation in the studied groups.

Discussion

It was of interest to evaluate the etiological factors associated with CP, in respect to the growing era of the genetic determinants of thrombophilia, in our developing country with a considerable prevalence of CP**.** In the present study, CP was more common in males than in females (٥٨٪ versus ٤٢٪; male: female ratio of ١.٣:١). These findings are in agreement with other studies, where males are more at risk of CP than females with a ratio of ١.٣:١[٨]. Recessive X-linked chromosome variants may contribute to this difference and males may be more vulnerable to genetic mutation[٩].

In the present study, the consanguinity was reported in ١٤ (٢٨٪) in the CP group and ٢ (١٠٪) in control group, with non significant difference. This finding is supported by the fact that consanguineous marriage in developing countries is highly frequent and encountered among the risk factors of CP[١٠].

In literature, there are many probable maternal and antenatal causes of white-matter damage and risk factors for CP[٨,١١]. In the present study, children with CP showed higher frequencies of maternal and perinatal risk factors including maternal infection, pre-eclampsia, multiple pregnancy, and maternal thyroid disease, but with non significant differences between both groups. These frequencies may not be accurate as it depends on obstetric history given by mothers in absence with data extraction from hospital maternal records or database, thus under-estimated or over-estimated data may be given by mothers.

In the present study, the frequency of children born pre-term in the CP group was significantly higher than that in control group (٢٦٪ versus ٥٪, P < ٠.٠٥). This finding is in agreement with other studies in literature which confirmed that preterm delivery is a major risk factor for CP[١٢,١٣]. The increased frequency of preterm children with CP may be attributed to the effect of improved neonatal intensive care management during recent years, leading to increasing survival of children born extremely preterm[٤].

In the present study, FVL mutations present in ٤٢٪ of CP children, ١٥٪ in control group, and ٤٣.٤٪ for all studied participants, with a statistically significant difference between CP and control groups. Our analysis indicates a high prevalence of FVL (١٥٪) in the normal controls however it is constant with the prevalence of FVL in related Arab general population which was reported to be ١٥٪ in Egyptians, ١٦٪ in Syrians, ٢٣.٥٪ in Jordanians and ٢٥٪ in Palestinians[١٤].

The high frequency of FVL mutation in both groups (CP and control) may be explained by high frequency of consanguinity in our study (١٠٪ in control, ٢٨% in CP group and ٢٣٪ in all), which constant with the reported high frequency of consanguineous marriage in Egypt up to ٣٥.٣٪[١٠]. In addition, our findings of the high frequency of FVL in children with CP may reflect the primary role of thrombophilia in the etiology of this neurological problem.

In literature, there are few case-control studies (Table ٤) aimed to describe a direct correlation between hereditary thrombophilia with regard to FVL mutation and CP but the results were controversial[١٥-٢٠]. However, the findings of our study are in agreement with Nelson et al.,[١٥] and Reid et al.,[١٨] which suggest that FVL mutation be considered as a risk factor for CP, in addition to other risk factors that are likely associated with brain injury.

In conclusion, the fVL mutation is one of a number of potential factors that may increase the likelihood of cerebral thrombo-embolism and subsequent CP in children. Further understanding of the risk factors involved in the development of CP may help in creating treatment modalities, such as anticoagulant treatment for mothers, in order to prevent this disability.

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