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ORIGINAL ARTICLE

Association of Location of Cyst with the family history and Mutated Gene among patients of Myelomeningocele

UROOJ FATIMA[1], SABAHAT GUL[2], IMRAN ISHAQ[3], SAHRISH MUKHTAR[4], IRAM QUDDUS[5], SYED HUSSAIN MEHDI[6]
[1]*Assistant Professor of Anatomy, Sind Medical College, Jinnah Sindh Medical University*
[2]*Associate Professor of Anatomy, Quaid e Azam Medical College, Bahawalpur.*
[3]*Professor of Anatomy, United Medical and Dental College*
[4]*Assistant Professor of Anatomy, Jinnah Medical and Dental College*
[5]*Professor of Anatomy, Sind Medical College, Jinnah Sindh Medical University*
[6]*Professor of Surgery, Jinnah Medical and Dental College*
Correspondence to Dr. Urooj Fatima, Email: urooj.fatima@jsmu.edu.pk

ABSTRACT

Aim: To determine association of location of cyst with the family history and mutated gene among patients of myelomeningocele.

Methodology: This cross sectional study consists of fifty diagnosed cases of myelomeningocele and ten healthy individuals taken as controls. The cases were collected from Jinnah Postgraduate Medical Center (JPMC) for a period of six months. The research was conducted in Dow Diagnostic and Research Laboratory (DDRL) (DUHS). Majority of the patients included in the study were neonates. Patients were examined for the presence and site of the cyst. Family history of the patients was also recorded. After taken informed consent from the patient's attendants, blood was drawn by a trained phlebotomist. DNA was extracted from whole blood followed by PCR amplification of VANGL1 gene.

Results: We found that among fifty individuals five patients showed mutation in VANGL1 gene. Mutation was absent entirely from the controls. The cyst was mostly (92%) present in the lumbar region. Only 2% patients showed positive family history. The association between site of the cyst and family history was statistically significant.

Conclusion: It is concluded that all mutation of VANGL1 gene was present at lumbar region.There was a strong relation between location of cyst and family history.

Keywords: Myelomeningocele, cyst, VANGL1 gene, PCR, mutation.

INTRODUCTION

Neural tube defects are a series of birth anomalies that comprises of cranial abnormalities and open or closed spinal lesions[1]. Closed spinal defects also called spina bifida occulta are concealed lesions with no obvious signs visible during physical examination[2]. Hence the patients usually have no symptoms and thus do not need any treatment. On the other hand, open lesions or spina bifida aperta are often apparent during physical examination as clear defects and consist of deformities like myelomeningocele (MMC)[3]. MMC mainly occurs due to partial closure of neural tube within 28 days of gestation. It would eventually lead to protrusion of a sac containing neural elements including meninges and cerebrospinal fluid[4]. It would lead to several neurological deficits which could lead to traumatic morbidities and various disabilities. Neurologic disability is mostly related to interruption in the growth of neural placode which would lead to sensory and motor deficit in the bladder/ bowel continence and also would result in lower limb paralysis[5]. Secondary neurologic deficit may lead to hydrocephalus[6], Arnold Chiari malformation[7] and scoliosis[8]. Furthermore, in long term surviving patients, the disability could be aggravated due to orthopedic problems[9] such as dislocated hips, foot or ankle deformities and joint contractures and tightness. The untreated open spinal dysraphisms have increase chances of infection and subsequent meningitis[10]. Thus the prognosis is usually worse if diagnosed late or left untreated. Studies have shown that the risk of NTD in the next pregnancies will be raised significantly if a mother had a previous child suffering from the disease[11].

Worldwide approximately 140000 cases of neural tube defects were reported[12]. The occurrence of spina bifida is about 0.5 of 1000 births globally and it is calculated in United States to be more than 3 per 10000 births[13]. The incidence of MMC is approximately 0.8 to 1 per 1000 live births worldwide[14] and in United States the prevalence is estimated to be 1in 3000 live births[15]. A study conducted in Northern China revealed that the recurrence risk in neural tube defects in subsequent pregnancies was 1.7% which was greater than in the United States[16]. On the other hand, the recurrence risk in myelomeningocele was reported to be 2 to 5% in the United States[17].

Multiple factors are involved in the etiology of myelomeningocele including maternal, environmental and genetic elements[18]. Maternal factors include low folate intake[19], alcohol consumption[20], usage of anticonvulsants[21], diabetes[22] andhyperthermia[23]. On the other hand, most of the cases of MMC are sporadic in origin. Various genetic factors might raise the risk of occurrence, like the presence of chromosomal defects[24] like trisomy 13 or 18 and individual with an affected twin or first degree relative. In animal models, particularly in mice, more than 40 genetic strains were found which were linked with spina bifida aperta. Whereas in humans' peculiar genetic mutations of amino-acid altering in the sequencing of coding regions such as planar cell polarity pathway genes and folate one-carbon metabolism encoding enzymes were published in some patients with spina bifida[25].

The purpose of the present study is to find out relation of location of cyst with family history and also with the mutated gene.

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MATERIAL AND METHOD

Across sectional study was conducted consisting of fifty diagnosed cases of myelomeningocele and ten patients taken as controls after approval from IRB. The patients were collected from Jinnah Postgraduate Medical Center (JPMC). Patients were examined for the presence of cyst. After performing clinical examination, family history of patients was taken. Most of the individual present in the study were infants so a consent form was signed by the parents. A trained phlebotomist was appointed to draw 2cc of blood. Further study was carried out in Dow University of Health Sciences (DUHS). DNA extraction was performed from whole blood spin column method. It was performed according to steps given by the manufacturer. It was followed by the PCR amplification of VANGL1 gene. PCR was carried out in a tube holding a reaction mixture of 20µl. The mixture was assembled by following constituents 500µM of four deoxynucleotides, 2 U of Taq polymerase (Promega), 10pmol of each forward and reverse primers for VANGL1 gene, 1.5 mM MgCl2 and 10xPCR buffer. The thermal cycler was designed to incubate initially the product for 10min for 95°C followed by 35 cycles comprising of 94°Cfor 30s. 64°C for 1min and 72°C for 1min with final extension for 10min at 72°Cfor 1min with final extension for 10min at 72°C. Then it was run on 2% Agarose and later on the amplified product was visualized under transilluminator. The length of the product was calculated according to migration pattern of a 50bp DNA ladder. The photograph of the product was taken with the help of DOC gel documentation system. The amplified products were of 404bp and were then sent for commercial sequencing. By aligning the sequence with the reference sequence, the mutation was analyzed. The amplified products were of 404bp and were then sent for commercial sequencing.

RESULTS

In the study it was seen that most of the patients forty-six (92%) have cyst on lumbar region followed by cervical (6%)and thoracic region(2%). Among fifty patients the family history was positive in one (2%) of the patient. The association between location of cyst and family history was highly significant, P < 0.001(Table-1)having Pearson Chi-Square value to be 15.986.

Table 1: Association between location of cyst and positive family history.

Family History	Location of cyst			n
	Cervical	Thoracic	Lumbar	
Negative	2	1	46	49
Positive	1	0	0	01
Total	3	1	46	50

P value 0.000

It was also seen that mutation in VANGL1 gene is found in five patients (10%). None of the controls showed mutation in the gene. The association between location of cyst and gene mutation is insignificant (Table 2) having Pearson Chi-Square value to be 0 .483.

Table 2: Association between location of cyst and gene mutation

Mutation	Location of cyst			n
	Cervical	Thoracic	Lumbar	
Negative	3	1	41	45
Positive	0	0	5	05
Total	3	1	46	50

P value 0.758

DISCUSSION

Literature has shown that MMC may run in families and being a multifactorial disease, genetics also plays an important role in the causation of the disease. In this instance, we assumed that in cases of MMC, the individuals having positive family history should have specific site of lesion. Also we hypothesize that if the gene is mutated as seen through different researches, the mutated gene samples should have some specific location.

In the study it was found that (92%) patients have cyst on lumbar region. De Faria TC et al in 2021 conducted a cross sectional study to detect the effect of anatomical level of lesion on bony malformations and functional motor skills in patients of myelomeningocele[26]. They found that in most cases the lesion was present at lumbar region. Holoyda et al in 2020 established the multilayered closure technique of lumbosacral myelomeningocele lesion in neonates and found that lumbar region is the most common site of defect[27]. Çetinkal et al in 2021 collected a data and found that lumbar region is the most common site of lesion[28].

It was also seen that (2%) of patients showed positive family history. The association of cyst with the family history was statistically significant but due to limited number of samples can't be conclusive. Dupepe[29] et al in2017 found that among patients of NTDs the general prevalence of family history was 16.9% of which 3.1% were seen in first degree relatives. The prevalence of MMC among all NTDs in terms of family history was 17.7% with 3.8% in first degree relatives. In maternal lineage, the family history was found to be 10.6% whereas in paternal lineage it was 8.7%.Ntimbani et al in 2020 found positive family history is present in only 5% of families, whereas the remaining 95% occur impulsively in women having no family history of disease. However, if a child had a positive family history the risk of the disease would increase to 3-8% if a woman had a previous child with MMC[30]. Piro et al in 2020 came across the fact if a parent has first child affected with the disease, the risk of second affected baby will be increased to 3-5 folds[31]. Iftikhar et al accepted the fact that mostly children having risk will increase to 1in 20 for next baby[32].

Among fifty patients of myelomeningocele, five patients showed mutation in VANGL1 gene V239F, V239G, V239S, V239I.Mutation V239I is present in two patients. In this study we found that all five mutations were present in individuals having cyst at lumbar region. Apparently it appears to be a significant finding but statistically is insignificant. Kibar et al in 2007 identified three mutations in the VANGL1 gene in patients with NTDs (V239I and R274Q and M328T)[33]. Bartsch et al in 2012 found 3 heterozygous missense mutations (c.518G 1 A, c.557G 1 A and c.613G 1 A) in VANGL1 gene in patients having NTDs[34]. Kibar et al in 2009 identified 10 missense variants in VANGL1gene[35].Tian Tian et al in 2021 established the fact that somatic mutations of planar polarity pathway (PCP) genes have a significant role in the causation of NTDs. He found three somatic mutations that were novel: CELSR1 p.Gln2125His, FZD6 p.Gln88Glu, and VANGL1 p.Arg374His[36].

CONCLUSION

Myelomeningocele is a life threatening condition. Results are consistent with the previous researches regarding the site of the cyst. The study was although limited by its sample size but contributed data to the literature that the site of the cyst is related to the family history of patient and also provide insight

finding that no matter if the gene is mutated it is not in any case related with the location of the lesion.

Conflict of interest: Nil

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