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Original Article

Experimental Autoimmune Encephalomyelitis (EAE) as a Potential MS Model

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Abstract

Introduction: Multiple sclerosis (MS) is one of the most serious syndromes in human populations. Although the source of MS is unknown, some patterns have been discovered according which the genetic background and environmental factors are the key elements affecting its development. Since human samples cannot be repeatedly drawn from the spinal cord, animal models have been the best options available for studying MS so far. Experimental autoimmune encephalomyelitis (EAE) is the most recognized model in this regard. This study aimed to study EAE on rabbits to correlate symptoms and lesions, deducing whether this really can be a suitable model for predicting the algorithm lying behind the MS disease.

Materials and Methods: For this purpose, 15 male two-month-old rabbits were divided into three groups, namely A, B, and C. The three groups were injected normal saline, complete Freund's adjuvant (CFA) + spinal cord homogenate, and normal saline + spinal cord homogenate via footpad and neck scruff, respectively. Then, they were submitted to laboratory and observed for histopathological changes.

Results: Group A did not show any signs or symptoms, while group B and C showed histopathological lesions. Moreover, group B was the only group showing clinical signs. There was also a significant difference between pathological and clinical signs in group A ($p<.05$), but not in group C ($p>.05$).

Conclusions: Considering the clinical and histopathological similarities between EAE and MS, the results suggest that EAE models are suitable to study MS.

Keywords: EAE, Rabbit, MS, Animal Model, histopathology

1. Introduction

Multiple sclerosis (MS) is a debilitating inflammatory disease of the central nervous system (CNS) affecting nearly 2.5 million people throughout the world [1, 2]. Due to the very complex nature of the disease, we are far from understanding the underlying variabilities; however, certain patterns have been detected [3]. First, the importance of genetic background has been revealed, with studies concluding that different genetic backgrounds will lead to different manifestations of an autoimmune T cell attack on CNS [4]. Also, environmental factors are as important [5]. In addition, some studies have shown that different CNS antigen specificity of the T cell response can involve different anatomical regions of CNS [6, 7]. All regions of CNS are prone to MS; yet it is mostly observed in white matter as focal regions of inflammation and demyelination [8]. Clinical signs of MS include gait ataxia, vertigo, tremor, and paresis [5]. Also, histopathological hallmarks include gliosis and demyelination of neurons in brain tissue [9].

MS is a sheer human disease and no other species have seen to develop it. Since conducting a human experiment is unachievable due to the impossibility of obtaining CNS tissue samples from individual patients repeatedly over time, animal models must be used to decipher the pathogenesis of the human disease [10]. There are several animal models for the MS mimicry, and each model has different merits and different levels of similarities with MS. Experimental autoimmune encephalomyelitis can be induced in most mammalian species, including humans. Whilst early examples of experimentally induced neurological disease were observed following vaccination with rabies virus infected CNS material [11], human EAE was more recently found when people with Alzheimer's disease were immunized by human beta-amyloid protein [12].

To date, experimental autoimmune encephalomyelitis (EAE) seems to be the best method available. Moreover, EAE is the most extensively studied animal model of MS. This model was first introduced by an American bacteriologist, Thomas Milton Rivers and his colleagues in the early 1930s [13]. Rivers et al. used normal brain extracts from rabbits and injected them into Rhesus macaques and showed that most of the monkeys developed acute CNS disease with immune cell infiltration and demyelinating lesions. No infectious agent could be cultured from the animals, putting to rest suspicions of an infectious etiology. Rivers' group also noted that the disease-inducing capacity of the brain extracts paralleled their myelin content, providing the first hint that myelin was involved in disease induction. Thus, the experimental autoimmune (then "allergic") encephalomyelitis (EAE) model was born [13].

Despite having a serious socio-economic impact on patients and societies (approximately $10 billion in United States) [14-16], no treatment is available. It is maybe the result of few clinical and histopathological studies, considering the

importance of the disease, for unraveling the pattern of the disease.

Although some studies have examined the clinical and histopathological aspects, there has been a dearth of research into evaluating the clinical signs to histopathological lesions, both simultaneously and in correlating manner. In this study, we aimed to put together both aspects and assess them at the same time to see whether EAE is the ultimate suitable model for MS. However, more studies are needed so that a better model will probably be developed or the ultimate pattern of MS can be revealed.

2. Materials and Methods

At the beginning of the study, a total of 15 healthy two-month-old male rabbits with a mean weight of 1.7 kg were used and a four-floor habitat cage was considered for maintenance. Prior to the introduction of subjects, all food-related equipment and levels were disinfected using Cetrimide (Sigma, USA) and 10% formalin. Then, subjects were broken down into three groups (A-C) on a random basis. Rabbits in group A received .5 ml normal saline via both footpad and neck scruff inoculation. For group B, 250 mg spinal cord with 5 ml complete Freund's adjuvant (CFA) were homogenized and .5 ml of the homogenate were injected via footpad and neck scruff route. Yet, in group C, 250 mg spinal cord was homogenized with 5 ml normal saline and then the subjects received .5 of the homogenate via footpad and neck scruff route. For the sake of simplicity, details of each group are presented in Table 1.

2.1. Necropsy

In the initial process of necropsy, an incision is made from the cranial part of the frontal lobe to the squamous temporal region, repeated on the other side as well. Two squamous temporal regions were connected by a latter incision, forming a triangle. The small ophthalmologic scissors were used to cut the vertebrae. Starting from the occipital bone level and then, with the tip of the scissors, alternating right and left side cuts of the vertebral bodies to progressively remove the vertebral arches. In this way, the spinal cord was uncovered in the vertebral canal. After that, both the brain and spinal cord were easily removed and submitted to histopathological analysis. All samples were stained with hematoxylin and eosin (H&E) dye for histopathological diagnosis of probable lesions.

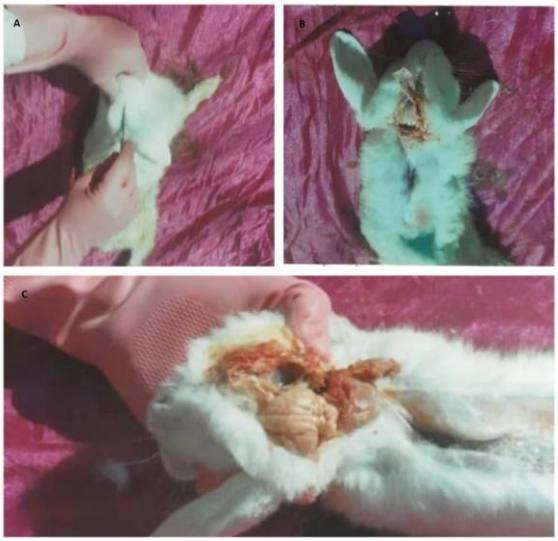

Figure 1. Separating brain from the skull; A. Initial incision from the upper part of the magnum foramen, B. Making the triangle with its base on magnum foramen, and C. Removing the brain of the produced triangle

Figure 2. Separating spinal cord from vertebrae

3. RESULTS

3.1. Clinical findings

To assess the clinical observations, the commonly accepted ascending grading scale of paralysis was used [17, 18] (Table 2).

Over the study course of 60 days, group A did not show any significant change. Unexpectedly, despite having high number of subjects, there were only two cases of clinical signs, with a score of 4 through 5, within the period of our study. In the morning of day 20, a subject from group B was paralyzed and died on day 21. On day 39, another subject from group B showed stiff muscle and paralysis and died on day 41. Brain, thoracic, and lumbar spinal cord were extracted and placed in 10% formalin for later histopathological assays. Formalin was replaced every 24 hours. On day 60, rabbits were euthanized using ether due to humane reasons. Twenty-four hours after the sample collection, they were submitted to a laboratory for slide preparation. It is worth noting that group C did not show any clinical signs.

3.2. Histopathological findings

In group A, no histopathological lesions, like their clinical results, were observed. As anticipated, vast areas of gliosis, demyelination, and lymphocytic meningitis were present at all subjects of group B in microscopic examinations and EAE was confirmed. Surprisingly, all subjects of group C were positive for both gliosis and demyelination as well as lymphocytic meningitis, unlike their clinical findings which were zero, and confirmed for EAE. There was a significant difference between groups A and B ($p < .05$). No significant differences were found in other relationships between groups ($p > .05$). The foremost likely cause of the discrepancy in group C is due to the limited time of the study. Histopathological results are shown in Fig. 3-5. Table 3 and 4 indicate the pathological, clinical, and the combined results of the two methods.

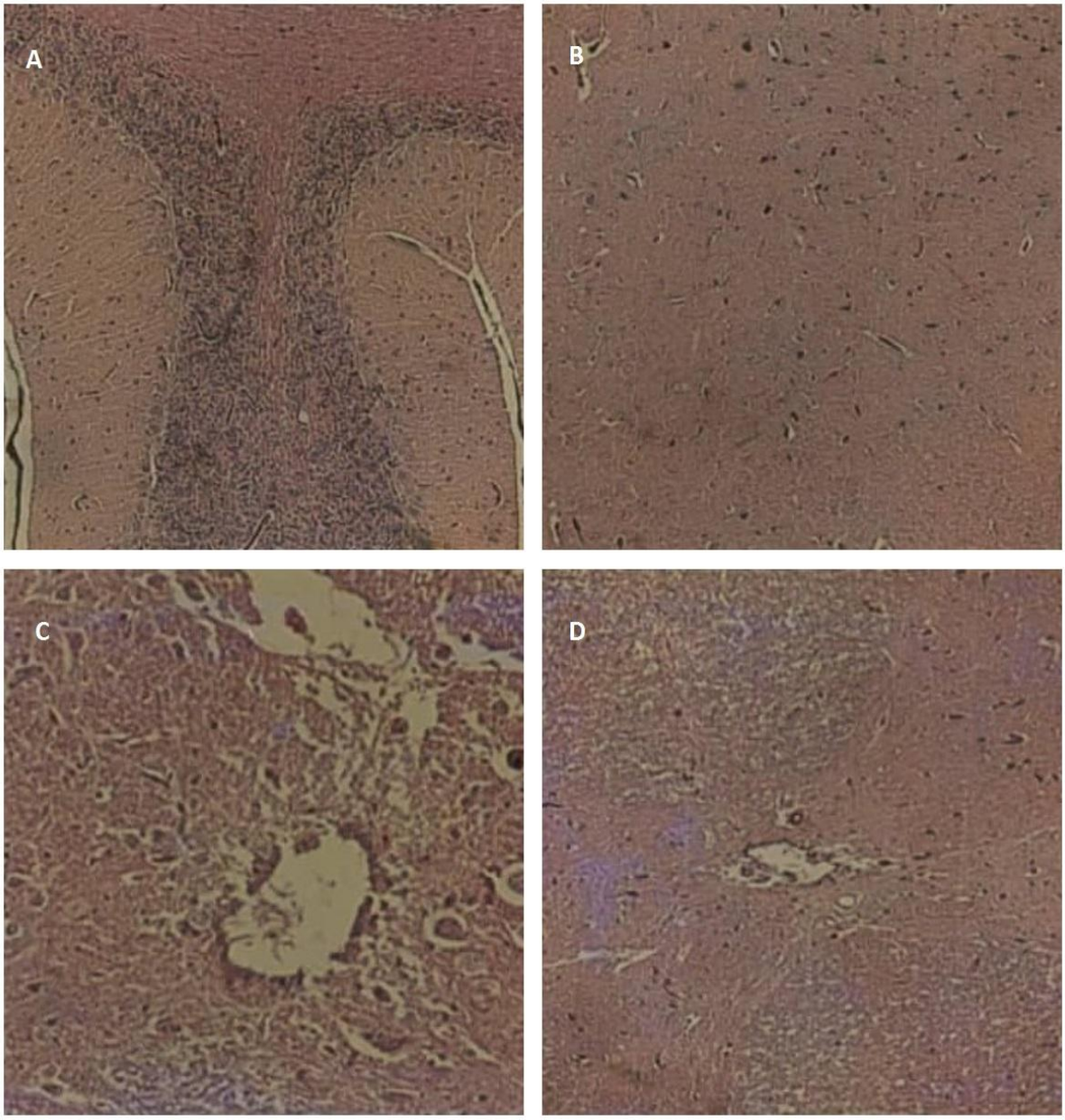

Figure 3. H&E staining of group A (control), all tissues are normal with no visible lesion; A. Cerebellum (40X magnitude), B. Brain (40X magnitude), C. Lumbar spinal cord (40X magnitude), and D. Thoracic spinal cord (100X magnitude)

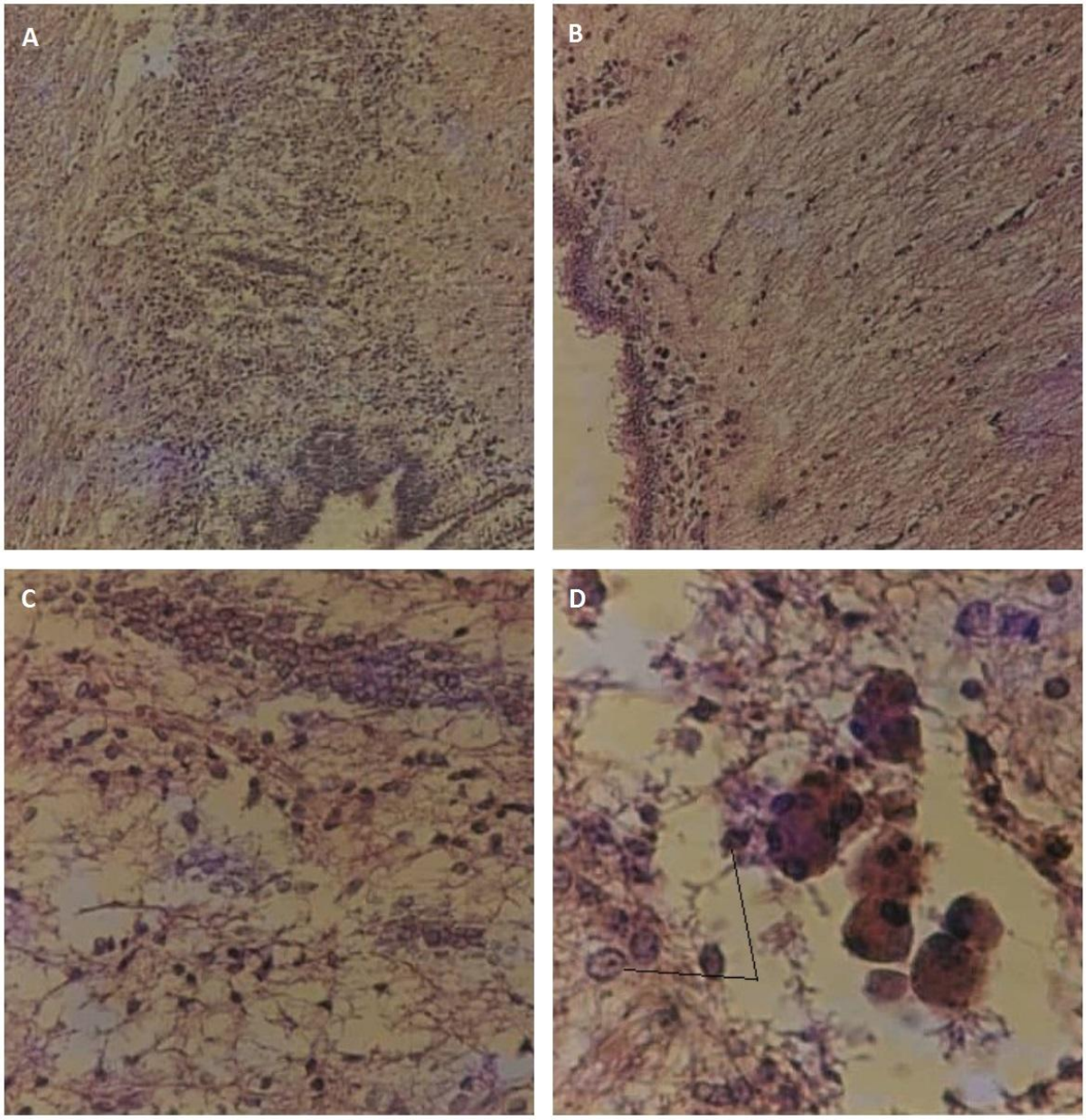

Figure 4. H&E staining in group B (CFA + spinal cord homogenate); A. Demyelination in brain ventricle (40X magnitude), B. Demyelination in brain tissue (100X magnitude), C. Demyelination in brain tissue (200X magnitude), and D. Macrophage in demyelinated region of brain (indicated with lines; 400X magnitude).

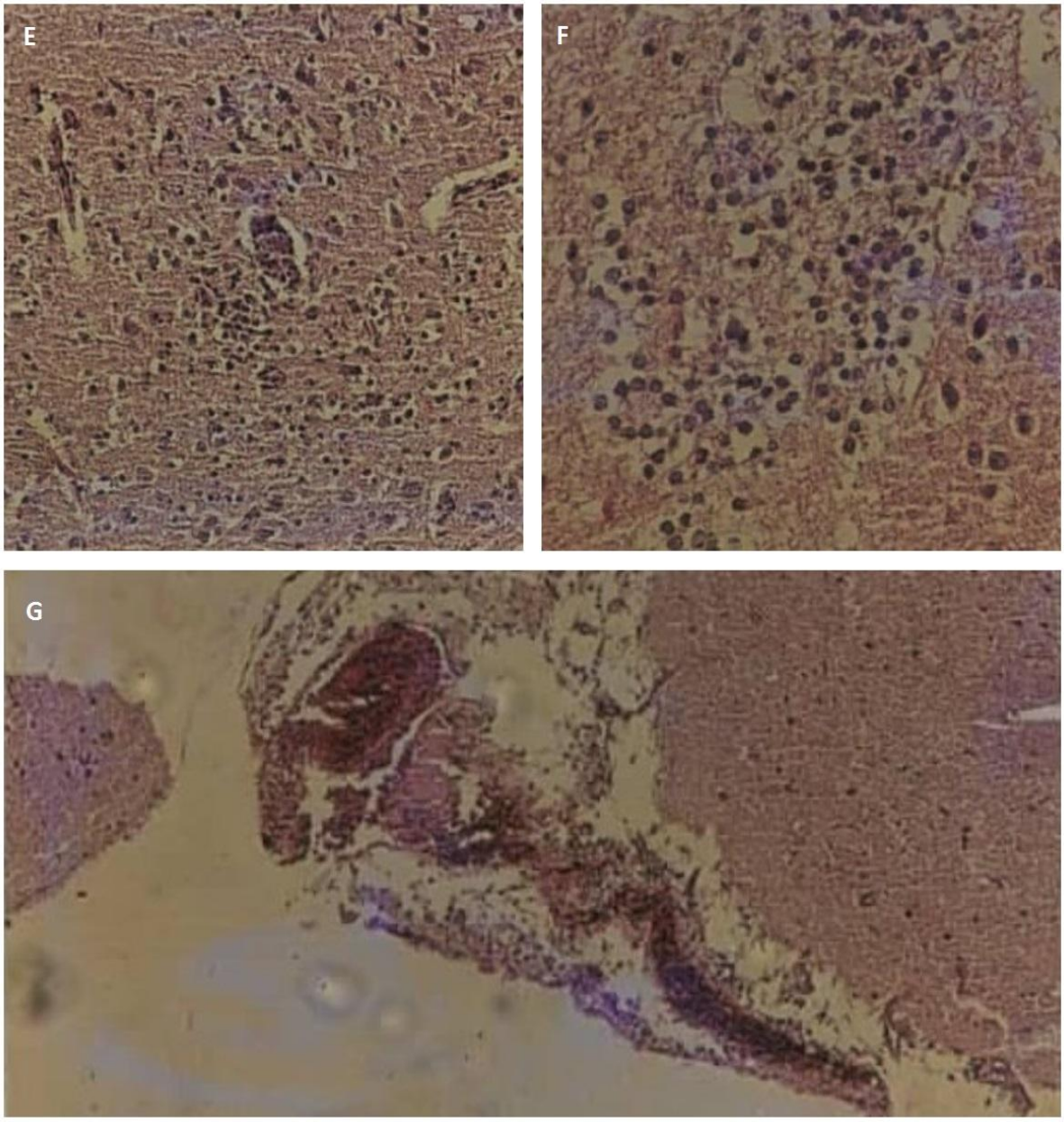

Figure 4 (continued). E. Gliosis in brain tissue (100X magnitude), F. Gliosis in brain tissue (200X magnitude), and G. Lymphocytic meningitis in brain tissue (100X magnitude)

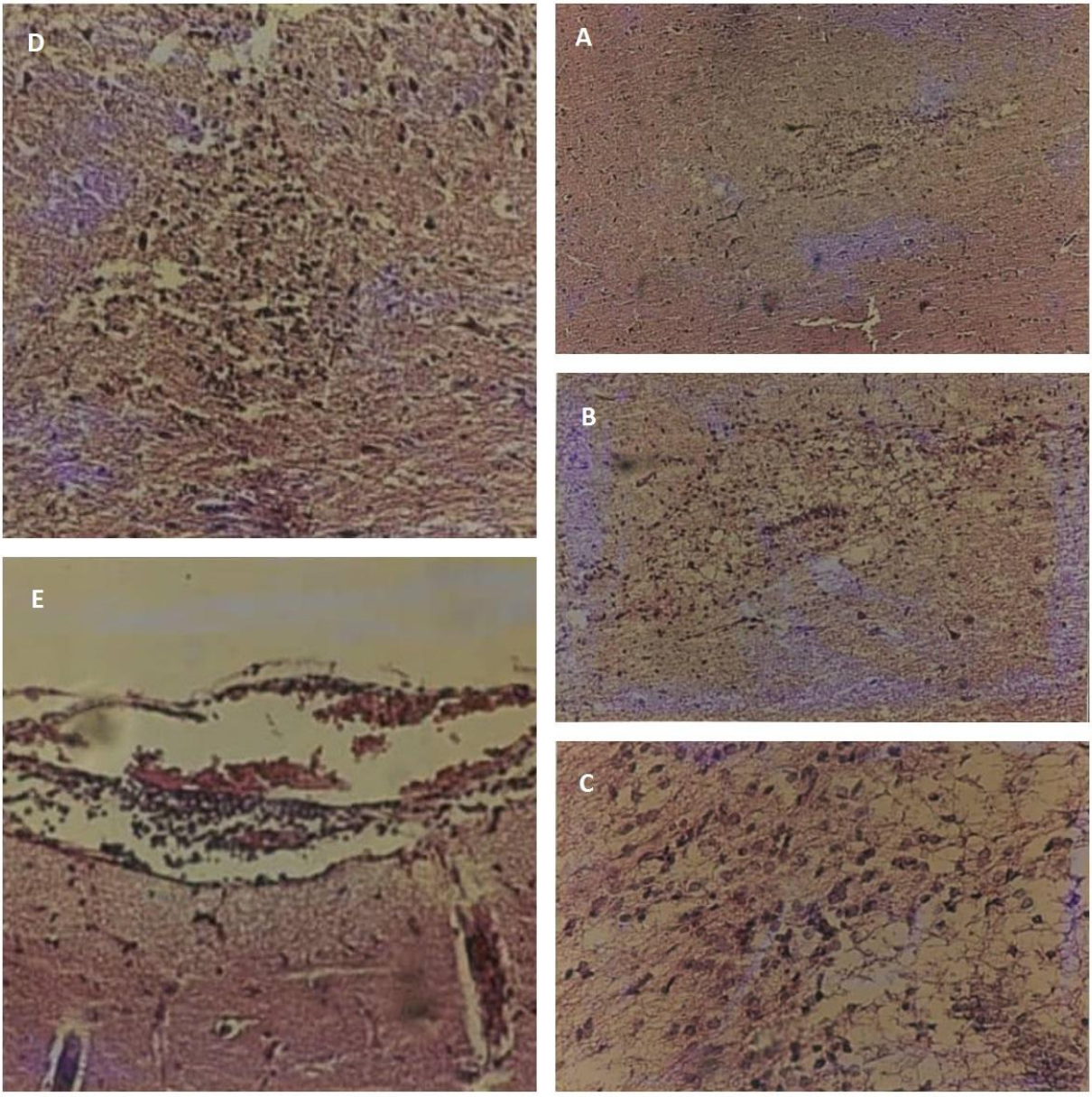

Figure 5. H&E staining in group C (normal saline + spinal cord homogenate); A, B, and C are demyelination in brain tissue with 40X, 100X, and 200X magnitude, respectively. D. Gliosis in brain tissue (100X magnitude), and E. Lymphocytic meningitis in brain tissue (100X magnitude)

4. Discussion

There were few studies available representing the experimental autoimmune encephalomyelitis (EAE) as an MS model, viewed in clinical and histopathological aspects of the disease, simultaneously. In this study, we compared our clinical and, more importantly, histopathological results with existing MS-based literature to test whether the EAE can be a genuine model for further studies. The results have strengthened our conviction that autoimmune encephalomyelitis is a proper MS model.

As anticipated, in the control group (group A), we observed neither clinical nor pathological abnormality. 100 percent of cases in group B, however, showed the pathologic hallmark signs and lesions of MS including demyelination of neurons and gliosis in the brain in addition to lymphocytic meningitis and macrophage infiltration, consistent with previous reports [6]. Also, 40 percent of rabbits demonstrated clinical signs including spasticity and paresis followed by permanent paralysis that even extended to death, having a number of similarities with Sinkjér et al. [19].

In group C which was injected with normal saline and homogenate spinal cord, we observed gliosis, demyelination, and lymphocytic meningitis, similar to group B. However, despite the pathological resemblance, no clinical signs or symptoms were observed. A major source of this discrepancy is most likely the limited time of our study.

To the best of our knowledge, very few experiments have been conducted so far to assess both clinical and pathological aspects of MS simultaneously. Particularly in recent years, there have been no experimental studies on EAE as an MS model. Since no better method than EAE has been recognized and no scientific method to cure MS has been developed, scientists should focus more on developing this method to hopefully find a way to treat the disease.

In various studies, delivering myelin-based proteins (MBP) altered peptide ligands acting as MBP ligands in people expressing MHC laptotypes and worsened the disease as in EAE [20-22], providing the most concrete evidence for the role of autoimmunity in people with MS. This concept was one of the main pillars of our study.

In the study of He et al., it was found that the B cell inhibition resulted in preventing the relapse in people with MS [23], whilst Weber et al. showed that B cell immunotherapy has an inhibitory action in EAE [24]. Additionally, T cells are required for the generation of antibody and antibodies take part in pathology, especially in demyelination occurring in EAE [10, 25, 26].

In 2012, Kuerten et al. collected the available data for EAE and compared it with MS pathology. They concluded that EAE can be a model of value for studying MS, which is consistent with our study [3]. In another study in 2015, Dendrou et al.

assessed the pathological and clinical signs and symptoms of MS models, concluding that EAE is a suitable model for MS [8] which is in line with our work.

While Glatigny and Bettelli concluded that we cannot expect an animal model to demonstrate all aspects of MS pathophysiology, it seems that EAE in animals including rabbits holds a great promise as a system to study MS pathogenesis in many aspects [27]. Moreover, Constantinescu et al. stated that EAE has contributed to the evolution and production of MS drugs, shedding some light on the pathogenesis of MS [28].

5. Conclusion

Due to the impossibility of sampling human spinal cord fluid regularly and complicated factors contributing to developing MS, animal models must be used; otherwise, clinical treatments of MS will continue to seem like a "look-see" practice. Given the obtained results in clinical and histopathological aspects of our research and comparing them with those of other studies, it is concluded that EAE can be used as a valuable model for MS mimicry. However, our data should be translated into the clinical situation to show how accurate our interpretations have been.

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Conflict of interest

The authors declare no conflict of interest.

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Tables:

Group	Specifications
Group A	0.5 ml normal saline
Group B	0.5 ml spinal cord + CFA homogenate
Group C	0.5 ml spinal cord + normal saline homogenate

Table 1. Group details

Score	Clinical findings
1	Loss of tail tonicity
2	Mild hind limb weakness
3	Partial hind limb paralysis
4	Complete hind limb paralysis
5	Complete hind limb paralysis with forelimb weakness or moribund

Table 2. Clinical scores

	Group	Gliosis in Brain	Demyelination	EAE
Pathological	1	0	0	0
	2	100%	100%	100%
	3	100%	100%	100%

	Group	Clinical Signs	Pathological Signs
Clinical	1	0	0
	2	40%	100%
	3	100%	100%

Table 3. Pathological and clinical results

Group	Clinical symptoms	Histopathological lesions
A	-	-
B	Hind limb paralysis + death	Demyelination, Gliosis, lymphocytic meningitis
C	-	Demyelination, Gliosis, lymphocytic meningitis

Table 4. Combined results