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Comparative Immunological and Histological Study of the Rat's Brain Infected with *Esherichaia Coli*

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Annotation: Background: Escherichia coli, an extra-intestinal infection, can break the blood-brain barrier (BBB) and cause serious neurological consequences such meningitis. Objectives: This study looks at how E. coli infection affects brain and visual structures in male and female rats, as well as sex variations in immunological responses, including interleukin-4 (IL-4) levels. Materials and Methods: E. coli was obtained from the Al-Hilla River using membrane filtration and injected into the eyes of 20 rats (10 male and 10 female). Results: After one month, the mice were dissected to extract brain and ocular tissues for histological investigation and IL-4 measurement using an ELISA. Hematoxylin and eosin (H&E) staining was employed to assess structural alterations. The study discovered no statistically significant sex-based variations in IL-4 levels or anatomical measures of the brain and eye in control groups. However, infection caused a substantial increase in brain and ocular diameters in both sexes ($p = 0.00$), most likely due to inflammation and immunological activation. Males had higher mean values for brain (129.62 vs. 117.98) and ocular (57.16 vs. 49.40) measurements in infected groups, but the difference was not statistically significant ($p > 0.05$). Infected samples showed structural damage, neuronal degeneration, and increased cellular infiltration, which supported the theory of infection-induced neuroinflammation. Conclusion: E. coli infection has a considerable influence on brain and visual tissues in both sexes because of inflammation. While men saw somewhat higher alterations, IL-4 levels did not differ considerably, indicating similar immunological responses. Larger research are required to investigate modest sex-related differences.

Keywords: Escherichia coli, eye, brain, IL-4.

Introduction

A multitude of microorganisms can cause brain disorders such as meningitis or even cerebral vasculitis. One of these organisms is Escherichia coli, which is an extra-intestinal pathogen, meaning it is capable of migrating from the intestines to other parts of the body where it can cause severe medical complications like meningitis [1]. Recent studies conducted in vitro and in vivo suggest that certain strains of E. coli are capable of penetrating the brain, with the most common route being through brain-infection-induced breakdown of the blood-brain barrier (BBB). In cerebrospinal fluid, there are E. coli strains from the clinically ill that have crossed the blood-brain barrier. They are one of the most important defenders of the central nervous system, which includes the blood-brain barrier (BBB) [2].

E. Coli is part of the family of bacteria called Enterobacteriacea and is one of the most significant reknowned bacteria [3]. Interleukin-4 (IL-4) is a key type of cytokine, mainly secreted by Th2 cells, mast cells, eosinophils and basophils. It is crucial in the management of immune responses by encouraging differentiation of naïve CD4+ T cells to Th2 cells, B-cell activation, and class switching to immunoglobulin E (IgE). IL-4 is considered to play a unique role in the development of humoral responses and responses related to allergy [4].

Materials and methods

In this work, *E. coli* was obtained from soil and injected into rats' eyes to reach their brains for a month. The animals were then dissected to remove the eyes and brain for histological sectioning and hemoginizing each one.

Isolation of Bacteria

Bacteria were isolated by using the standard membrane filtration technique (USEPA, 2000). The water sample was collected from Al- Hilla river. Serial dilution was made (10-1 to 10-5) ready and mixed well, (10ml) of water sample transfer to (90 ml) of normal saline after that filtered through a cellulose acetate membrane sterile 0.45 µm using vacuum filtration system and transfer cellulose filter paper on to chromogenic agar and incubated at 37 °C, each sample was examined in triplicated.

Animals

Twenty rats (10 male and 10 female), 5 male and 5 female infected with *E. coli* through eye, and the left were control. All animals were dissected to abtain eye and brain, each one cut in two parts (one kept in formadehyde 4% for brain).

Histological section

All of sections flooded in alchol in different concentrations then sectioning and stainning with heamatoxlin and eosin. This sections exame at 40X.

Interlukin _4

In all homogenized samples were determened Il_4 by using ELISA kit (Elabscience).

Statistical analysis

the result have been used used SPSS statistical program version 26.

Ethical approval

This study approved by local ethics committee of colloge of science /Babylon university under number of decument 3048, 15/4/2024.

Results

Shows a substantial level of structural disorder. There is an increase in cellular infiltration, which suggests inflammation. alteration of the brain's natural structure, maybe caused by bacterial invasion and immune response activation. The tissue displays evidence of neuronal injury, indicating that the bacterial infection induced histopathological alterations. (B) has a fairly regular layout and well-preserved neuronal architecture. The lack of major histological alterations. In (A), the tissue has a normal structure (figure 1).

The histology section shows that the cerebrum tissue of male control and E. coli-infected samples varies structurally. The (B) shows signs of tissue injury, such as potential neuronal degeneration, vacuolation, and structural abnormality. The more stable and preserved tissue structure in (A) indicates that the bacterial infection in (B) resulted in histopathological alterations (figure 2).

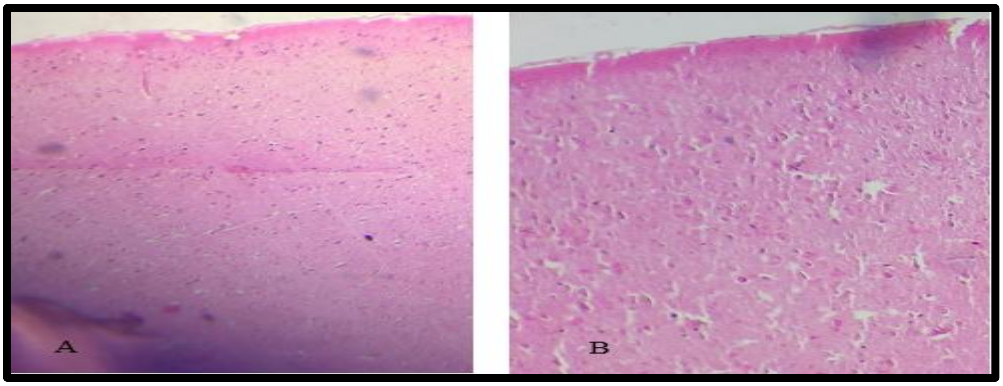

Figure 1. Cerebrum's longitudinal section of female, A: infection cerebrum, B: normal cerebrum stained with H&E,40X.

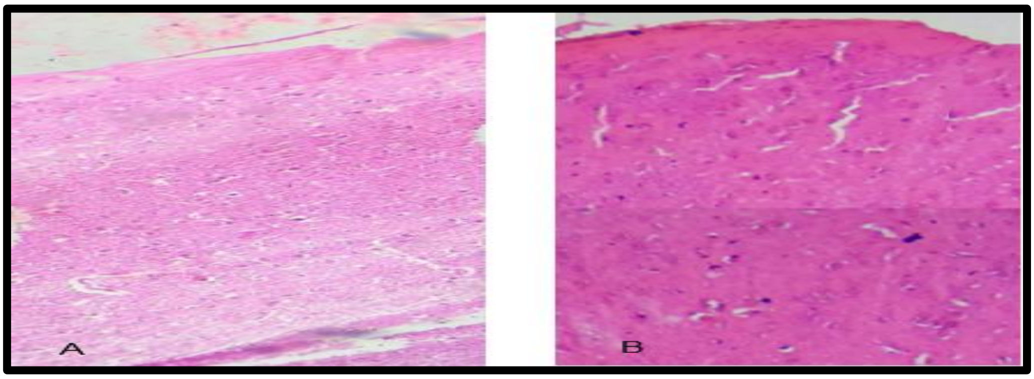

Figure 2. Cerebrum's longitudinal section of males, A: infection cerebrum, B: normal cerebrum stained with H&E,40X.

The current research looked at differences between male and female groups for two variables, "brain" and "eye," using their mean values and associated standard errors (SE). The p-values for both variables indicate no statistically significant differences between the sexes (table 1).

Males had a higher mean value (129.62) than females (117.98), indicating a possible tendency toward bigger brain measures in males. However, the p-value (0.456) suggests that this difference is not statistically significant, implying that any observed variance is more likely attributable to random chance than a real biological difference. Males also have a higher mean eye measurement (57.16) than females (49.40), but the p-value (0.270) suggests that this difference is not statistically significant. The standard error for males (6.00979) is notably larger than for females (1.39284), indicating greater variability in the male group. (table 2).

The offered data contrasts male and female groups on two variables, "brain" and "eye," under two conditions: infection and control. The means, standard deviations (SD), and p-values all show statistically significant differences (p = 0.00) between the infected and control groups in both sexes. An explanation of the findings is provided below, along with supporting references (table3).

Table 1. Compare IL-4 levels in typical male and female brains and eyes.

Group		Mean ± SE	P – value
brain	Female	8.0000±0.89443	0.776
	Male	7.6000± 1.02956	0.776
eye	Female	6.8000±0.89443	0.814
	Male	6.8000±1.92354	0.813

* Significant differences P > 0.05

Table 2. Show IL-4 disparities in infected brains and eyes between males and females.

Group		Mean± SE.	P – value
Brain	female	117.9800±9.83526	0.456
	male	129.6200±11.13433	0.455
Eye	female	49.4000±1.39284	0.270
	male	57.1600± 6.00979	0.243

* Significant differences P > 0.05

Table 3. Show variations in IL-4 levels between male and female brains and eyes following bacterial infection.

Group		Mean ± SD	P - value
Brain male	Infection	129.6200 ± 11.13433	0.00

	Control	7.6000± 1.02956	
Brain female	Infection	117.9800±9.83526	0.00
	Control	8.0000±.89443	
Eye Female	Infection	49.4000±1.39284	0.00
	Control	7.2000± .86023	
Eye Male	Infection	57.1600± 6.00979	0.00
	Control	6.8000± 1.39284	

[*] Significant differences P < 0.05

The mean value for females (8.0000 ± 0.89443) is somewhat higher than that for males (7.6000 ± 1.02956), but the difference is not statistically significant (p-value = 0.776). Both male and female groups had the same mean value (6.8000), however men have a larger standard error (1.92354) than females (0.89443). The p-value (0.814) supports the absence of significant sex differences in this measure.

Discussion

This conclusion is consistent with prior study, which found that while there may be morphological or physiological differences between male and female brains, these differences do not always transfer into statistically significant differences in functional or cognitive assessments [5]. According to research, brain size disparities are primarily explained by overall body size rather than sex-specific neurobiological variances [6].According to research, while there may be slight sex variations in ocular components such as corneal thickness and retinal nerve fiber layer thickness, these differences are seldom statistically significant across groups [7][8].This conclusion is consistent with previous study, which found that male brains had a higher absolute volume due to variations in body size [6]. However, research indicate that brain shape and function do not always correspond with cognitive capacity, since male and female brains are very comparable in terms of connection and functioning [5].

Previous research on ocular differences has indicated that males had bigger ocular dimensions, such as axial length and corneal diameter, than females [8]. These changes, however, are often modest and have no significant impact on visual acuity or function. The lack of statistical significance in this dataset aligns with findings that, despite some anatomical distinctions, sex-based differences in eye structure are not always pronounced [9]. The considerable rise in brain measures in the infection group compared to the control group indicates that infection has a significant impact on brain anatomy. Infections can induce inflammation, edema (swelling), or structural changes in the brain, resulting in an increase in measured size [10]. Neuroinflammatory reactions to illnesses such as bacterial meningitis, encephalitis, or parasite infestations have been shown to cause brain swelling, which might explain the observed discrepancies [11].The considerable rise in eye measures in the infection group indicates that infections may be producing pathological changes such as edema, increased intraocular pressure, or other structural abnormalities in the eye. Infections such as conjunctivitis, uveitis, and parasitic infections (e.g., Toxoplasmosis, Onchocerciasis) can cause ocular inflammation and increased eye size [12]. The disparity in male and female eye measures in the infection group might be attributed to differences in immune responses and susceptibility to infections. According to studies, males frequently have a more exacerbated inflammatory response than females, which may explain why infected males have a bigger rise in eye size.

The greater levels in infected men compared to infected females might be attributed to sex-based immune responses. According to research, males and females react differently to infections owing to hormonal and genetic variations, which influence the intensity of inflammation and its consequences on the brain [13][14][15].

Conclusion

These results show no significant sex differences in brain and eye measurements, a finding consistent with several studies. However, they also indicate that while biological sex may alter anatomy, the differences are not substantial. Nevertheless, it cannot be ignored that infections lead to a significant increase in brain and eye measurements, possibly due to inflammatory processes. These findings are further explained and supported by sex-linked immune responses, which exhibit differences between males and females.

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