

Investigation of biomarkers associated with intestinal permability in patients with migraine

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Our agenda for today



- 1 Aim of the study**
 - Objectives of the study
 - Overall limitations

- 2 Migraine and its burden on society**
 - Definition of migraine
 - Prevalence and the burden on society

- 3 Discussion on gut-brain axis**
 - Definition and functions

- 4 Zonulin protein and LBP**
 - Use of zonulin protein to assess gut permeability
 - Use of LBP to assess gut permeability

- 5 Methodology**

- 6 Results**

- 7 References**

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1. Aim of the study

Aim of the study



To investigate the relationship between migraine and gut permeability through non-invasive biomarker quantification

Our aim for today is to;

1. Detect whether any correlation(s) between migraine and our parameters exists
2. If there are any, we wish to seek the change of identified correlations between migraine sub-groups

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2. Migraine is a prevalent headache disorder with a huge burden on society

Migraine is...

- 1 A primary headache disorder
- 2 Lasting between **four to seventy-two** hours per attack
- 3 Sometimes accompanied by **phonophobia, photophobia, and/or vomiting**
- 4 Preceded by a reversible **visual or sensory aura**
- 5 A headache with **moderate or severe pain** localized on one side or behind eyes, **which may pulsate**



...quite prevalent and creates a huge burden on society

%18.9 | Prevalence among women¹

%9.8 | Prevalence among men¹

In 2019, a study² with 13,064 correspondents demonstrated...

%32.7 | People with migraine thinking headaches have affected their carrier



%49 | People with migraine reporting that they would be a more suitable partner without migraine



\$34.2 bn | Estimated financial burden of migraine on US healthcare system³



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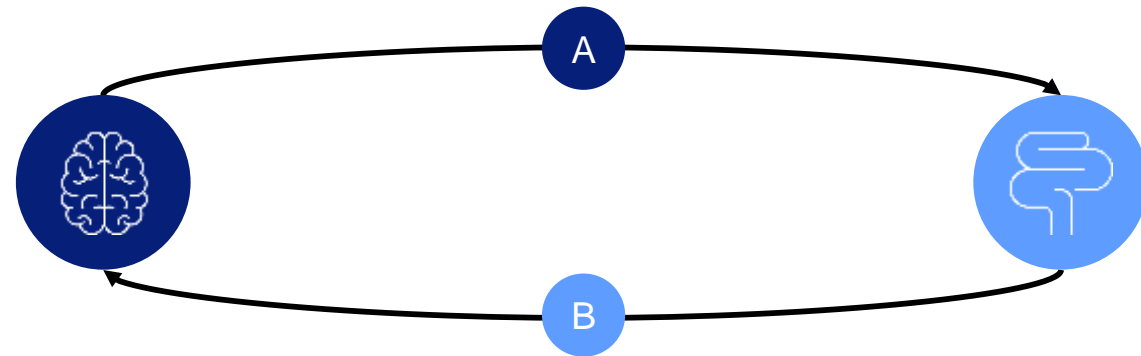
- 7 References**

3. «Gut-brain axis» refers to the mutual interaction between the central nervous system (CNS) and the gastrointestinal system (GI)

Gut-brain axis...

...as illustrated below ⁴⁻⁶

- 1 Tracts movements and functions (**sensory and secretory**) that are normally regulated by the brain
- 2 Affects several brain functions, including behavior, cognition, and even nociception
- 3 Has been linked to disruption of **neurological conditions** such as multiple sclerosis, mood and anxiety problems, **Alzheimer's disease, Parkinson's disease, and migraine**⁴⁻⁶



A

Central nervous system (CNS) modulates gut microbiota through:

- Sending efferents of sympathetic and parasympathetic systems
- Releasing neuroendocrine factors
 - Adrenal cortex and medulla

B.1

Indirect signaling:

- Microbiota derived neurotransmitters
- Inflammatory molecules (cytokines)
- Hormones

B.2

Direct connection

- Stimulating end terminals of the vagus nerve

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4.1. Zonulin protein and LBP help us to assess the gut permeability

☐ Detailed next

1 Zonulin protein

2 Lipopolysaccharide-binding protein (LBP)

Function

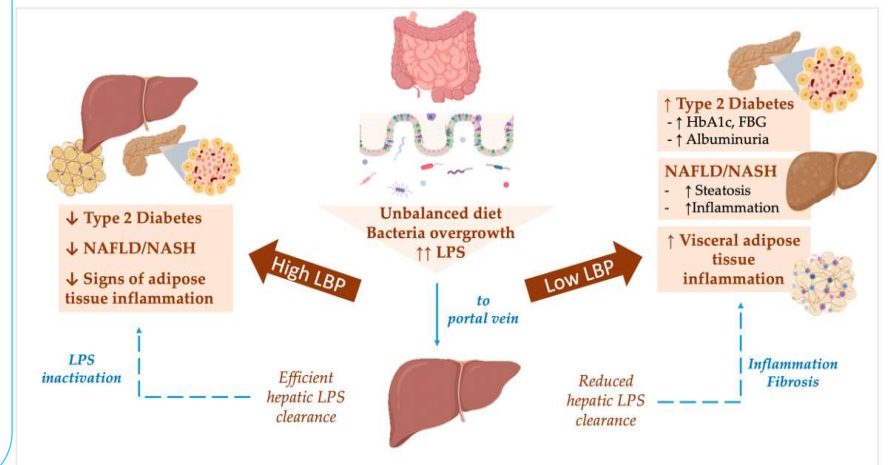
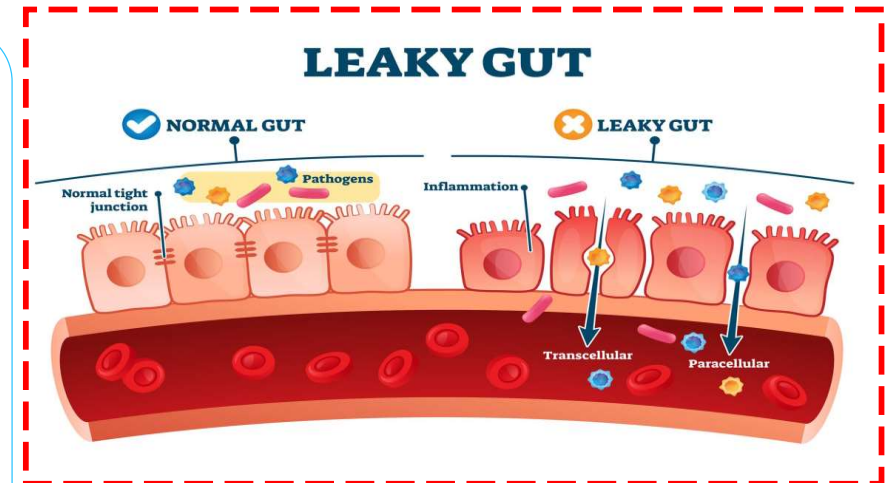


The 47 kilodaltons (kDa) protein zonulin, is involved in intestinal innate immunity and promotes intestinal permeability in the small intestines⁷

It is produced by the epithelium of the small intestine in humans in response to external stimuli.

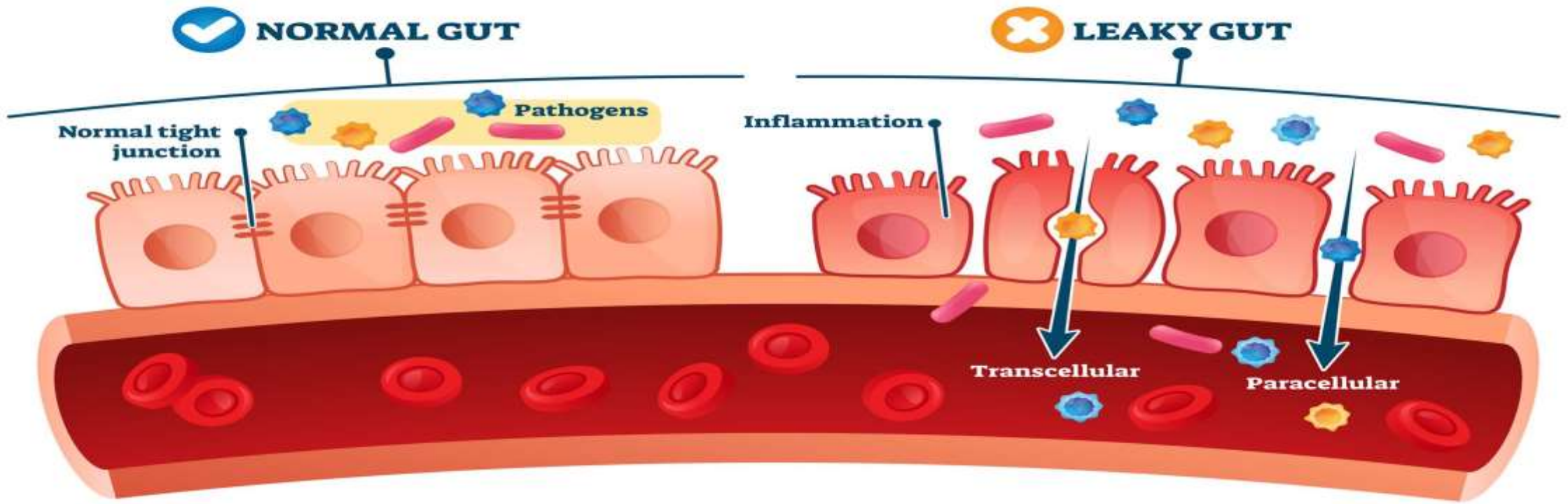
By binding to certain receptors on the epithelial surface of the distal ileum and jejunum, zonulin affects the function of tight junctions (TJs) in cells and binds actin fibers to one another through protein kinase-c (PKC)⁸⁻⁹

Zonulin disassembles and increases the permeability of TJs by transactivating the epidermal growth factor receptor through the proteinase-activated receptor-2. Zonulin controls TJs, which in turn controls the transport of paracellular antigens¹⁰



4.1. Zonulin protein and LBP help us to assess the gut permeability

LEAKY GUT



4.2. Zonulin protein and LBP help us to assess the gut permeability

Detailed next

1 Zonulin protein

2 Lipopolysaccharide-binding protein (LBP)

Function

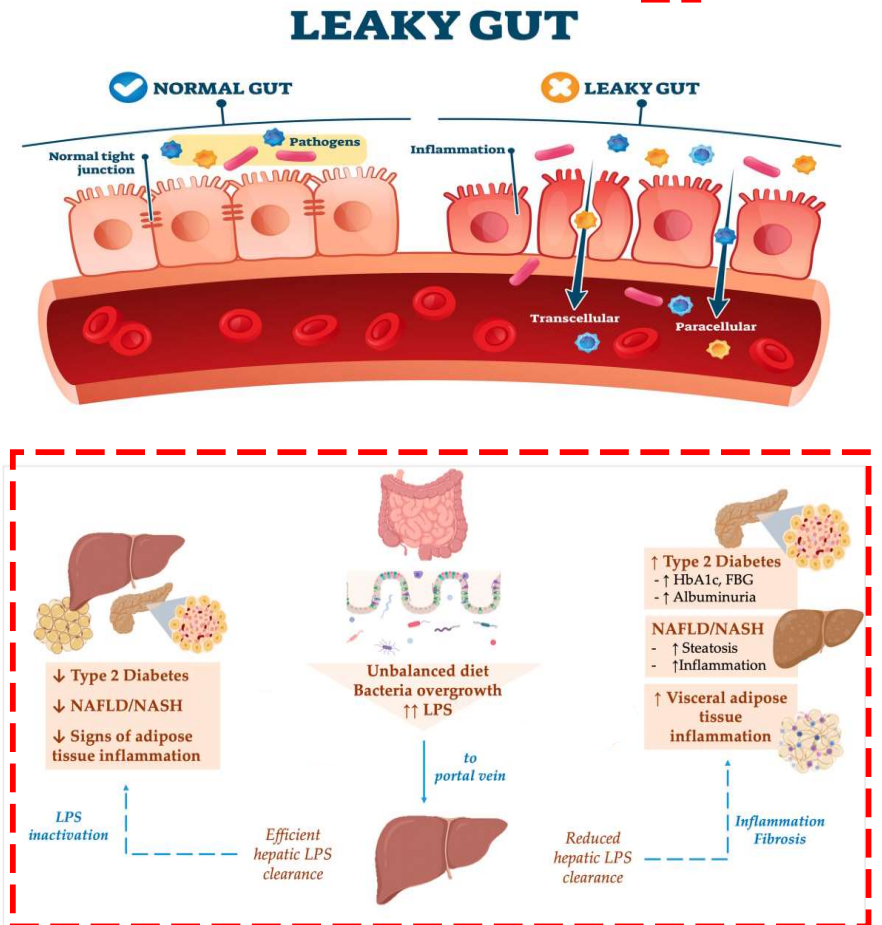


Plasma lipopolysaccharide-binding protein(LBP) is another protein that involve in gut permeability.

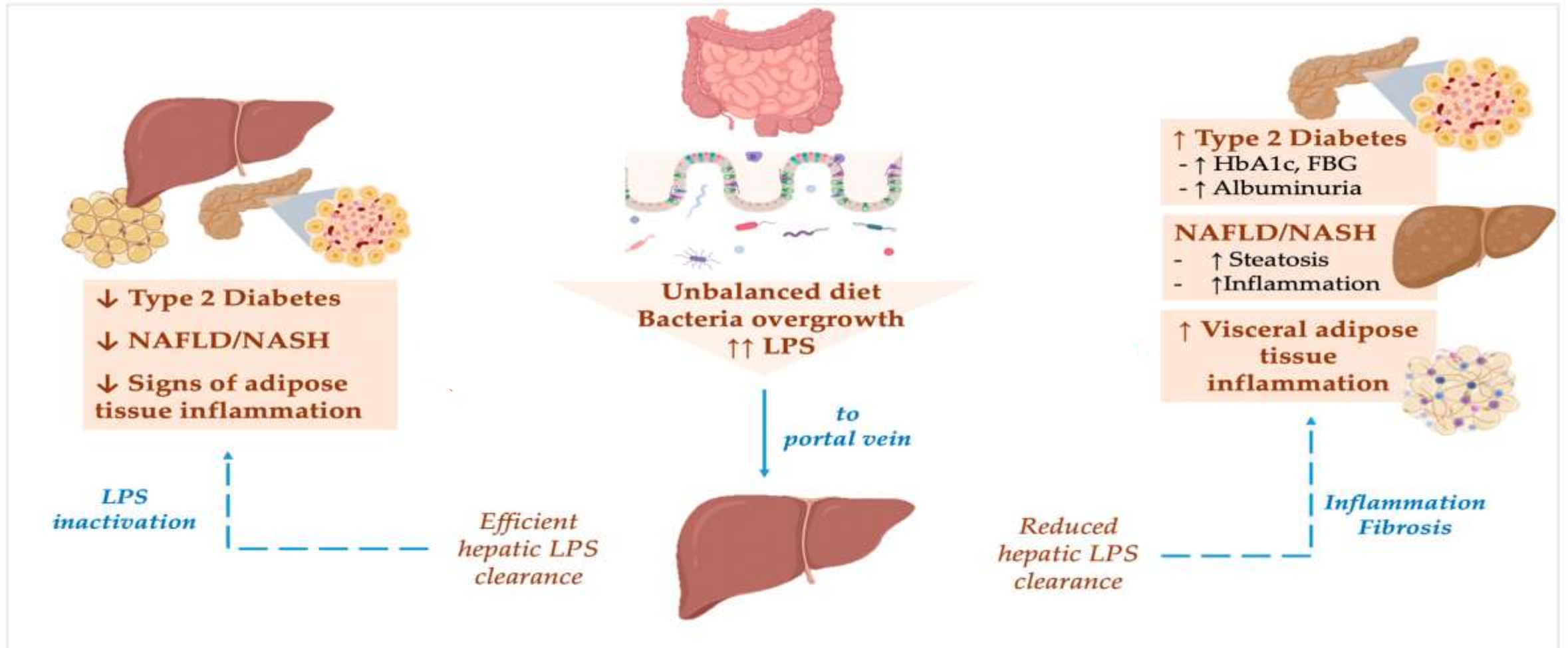
To evaluate the state of chronic endotoxemia and the immune system's reaction to it, LBP, an endogenous protein that binds to LPS and transfers LPS monomers to "Cluster of Differentiation 14" (CD14), has been utilized as a substitute.¹¹⁻¹² According to recent papers, LBP concentrations may be linked to obesity, high-fat diets, and chronic illnesses.¹¹⁻¹⁶

Hepatocytes produce lipopolysaccharide binding protein (LBP), an acute-phase protein that circulates in the blood. Because LBP binds to bacterial lipopolysaccharides, which are partially derived via intestinal translocation, it is thought to be a biomarker of intestinal permeability as well as an indicator of endotoxemia¹⁷

A study showed a correlation between plasma LBP levels and lac/man ratio implying that LBP being a promising biomarker to assess gut permeability



4.2. Zonulin protein and LBP help us to assess the gut permeability



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5. Our methodology is comprehensive and includes various dimensions reflects our study's aim

Sample collection methodology and criteria

	Migraine Patients (n=50)	Control Group (n=30)
Inclusion Criteria	18-65 years old Migraine Patients	No medical history of any type of inflammatory diseases
Exclusion Criteria	<ul style="list-style-type: none">-Diseases that effect GI tract such as but not limited to; Crohn, IBD, Celiac's, Ulcerative Colitis-Other pathologies that effect CNS-Oto immune diseases-Bacterial infection	<ul style="list-style-type: none">-Diseases that effect GI tract such as but not limited to; Crohn, IBD, Celiac's, Ulcerative Colitis-Other pathologies that effect CNS-Oto immune diseases-Bacterial infection

5. Our methodology is comprehensive and includes various dimensions reflects our study's aim

■ Detailed next

How did we collect samples?

We have collected **51 migraine patients** diagnosed according to **International Classification of «Headache Disorders» (ICHD-3)** criterias

We have collected 30 healthy specimen proportionally in line with 51 patients' **sex and age status**



How did we study samples?

The biochemistry lab located in our university's facility led by Şahabettin Selek, MD and his team used commercially available enzyme-linked immunosorbent assay (ELISA) to measure aforementioned parameters in plasma serum



How did we analyze samples?

Continuous numerical data will be expressed as mean \pm standard deviation (lowest to highest values). Non-continuous numerical data will be presented as median (lowest - highest values). Categorical data will be expressed as numbers (percentage)

Numerical data will be shown as mean \pm standard deviation or median (range), and categorical data as numbers (%). Group comparisons: chi-square test for categorical, Shapiro Wilk for numerical normality, Independent Samples t-Test or Mann-Whitney U for numerical (based on distribution). For repeated measures, rmANOVA or Friedman test (parametric/non-parametric), with post-hoc Dependent Samples t-Test and Bonferroni correction

5. We have used 10 sub-groups to further our analysis

 Detailed next

Patients' sub-groups

1
Nausea

2
Vomiting

3
Dizziness

4
Aura (type)

5
Photophobia

6
Sonophobia

7
Onset time

8
Pain duration

9
Pain frequency

10
Pain intensity

Background

We also sub-grouped patients to various dimensions such as **nausea, vomiting, dizziness, aura (type), photophobia, sonophobia, onset time, pain duration per hours, treatment status, pain frequency per month and pain intensity.**

For **pain frequency**, the sub-group has been further separated based the frequency of their migraine attacks per month ($n \geq 8$ or $n < 8$)

On the other hand, **Visual Analogue Score (VAS)** has been used to identify pain intensity

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Treatment Status

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6. Our results

Pain frequency

	zonulin	lbp
Mann-Whitney U	240	204.5
Wilcoxon W	516	480.5
Z	-1.552	-2.224
Asymp. Sig. (2-tailed)	-0.121	0.026

	frequency	N	Mean rank	Sum of Ranks
Zonulin	1	28	28.93	810
	2	23	22.43	516
	Total	51		
lbp	1	28	30.2	845.5
	2	23	20.89	480.5
	Total	51		

Pain intensity

		Zonulinlevel
VAS	Correlation Coefficient	0.403**
	Sig. (2-tailed)	0.003
	N	51

Results

There are **no meaningful differences between migraine patients and healthy control group** in terms of levels of our parameters ($p > 0.05$)

However, for **pain frequency and pain intensity groups**, there is **mildly strong correlation between our parameters**. ($p = 0.026$ for LBP and $p = 0.003$ Zonulin respectively)

6.2 Discussion

- Our data suggest a correlation between Zonulin levels and pain intensity while another correlation between LPB levels and pain frequency. Further studies are needed to elaborate the connection between migraine and disrupted intestinal permeability.
- Still, there are some speculations whether patient's unhealthy diet trigger their migraine.

Strong Sides



First study to investigate such parameters in migraine patients as to our literature review



Our healthy specimen match our patient group proportionally in means of age and sex

Limitations



Limited funding for the study given that it is a student project



Sensitivity and susceptibility of commercially available ELISA kits raises doubts



Given time limitations, sample collection process has been difficult and could have been improved

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7. References

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Next Steps

- Another research can be conducted using a bigger sample with more parameters
- Another research consisting a gluten-free and high fiber diet recommended to severe symptomatic patients to observe their pain intensity and frequency.

SEX	AGE	main frequency/month	duration/hours	onset time	VAS	nausea	vomiting	dizziness	aura	prodrom	nsory, 3-motor, 4-aphasi	Photophobia	Sonophobia	Zonulin I	LPBP level	First Diagnose/Treatment taken 0:FD 1:TT	
F	24	2	96	12	7	1	1	1	0	0	0	0	0	0	27,203	18,6747	1
F	31	2	12	4	8	0	0	0	0	0	0	0	1	1	17,428	9,0904	1
F	34	2	24	3	7	0	0	0	1	0	2	1	1	1	10,802	7,5972	0
F	35	8	60	7	5	0	0	0	1	0	1	1	1	1	21,183	14,672	1
M	63	8	24	40	4	0	0	0	0	0	0	0	0	0	11,863	6,5328	0
F	50	2	84	20	9	1	1	0	0	0	0	1	1	1	12,73	7,1547	0
F	43	2	12	4,0ca	4	0	0	0	0	0	0	0	1	1	9,1287	4,5433	0
F	35	8	48	10	8	1	1	0	1	0	1	1	1	1	73,716	44,641	1
F	28	20	24	7	5	1	0	1	1	0	1	1	1	1	65,631	46,465	1
F	51	2	72	2	6	1	0	0	1	0	1	1	1	1	37,844	20,816	1
F	41	5	12	20	4	1	1	0	0	0	0	1	1	1	12,997	6,2482	1
F	30	6	24	10	3	1	0	0	1	0	1	0	0	0	13,211	7,0859	0
F	35	4	72	2	4	1	0	1	1	0	2	1	1	1	10,261	4,0339	1
M	34	12	24	6	5	1	0	0	0	0	0	0	1	1	10,637	11,433	0
F	41	17	12	12	6	1	0	0	0	0	0	0	0	0	11,51	5,529	1
F	39	10	72	8	3	1	0	0	0	0	0	1	1	1	21,298	5,7623	0
F	21	8	24	3	4	0	0	1	0	0	0	1	1	1	21,933	13,41	0
F	55	4	72	20	3	1	0	0	0	0	0	0	0	0	12,413	6,639	0
F	40	4	48	10	5	0	0	1	1	0	2	1	1	1	15,148	10,234	1
M	39	15	24	2	8	0	0	0	0	0	0	1	1	1	14,601	5,7613	0
F	22	4	2	4	6	0	0	0	1	0	1	0	0	0	14,525	7,7442	0
F	28	4	24	10	8	1	0	0	0	0	0	1	1	1	48,282	29,615	0
F	18	10	24	3	7	0	0	0	0	0	0	1	0	0	7,8322	5,2576	0
F	49	10	12	3	6	1	0	0	0	0	0	1	1	1	11,782	7,0311	1
F	57	10	24	40	8	1	0	0	0	0	0	1	1	1	14,125	10,808	0
F	53	14	24	20	4	1	0	0	1	0	1	1	1	1	17,924	9,1626	0
F	26	6	36	4	5	1	0	0	0	0	0	1	1	1	9,3262	4,3073	0
M	24	2	24	5	6	1	0	0	0	0	0	1	0	0	48,808	27,904	0
F	25	10	72	10	7	0	0	0	1	0	1	1	0	0	28,598	15,006	0
F	31	2	48	5	3	1	0	1	0	0	0	1	1	1	13,215	5,9056	1
F	45	12	4	26	3	0	0	0	0	0	0	0	0	0	7,0295	4,0258	1
F	36	2	24	15	3	1	1	0	0	0	0	0	0	0	8,8227	4,6106	1
F	40	10	60	25	4	1	1	0	0	0	0	0	0	0	8,5973	5,0493	1
F	33	6	72	20	6	1	1	0	1	0	1	1	1	1	8,6905	4,1249	0
F	21	4	24	9	3	0	0	0	0	0	0	1	1	1	11,21	4,8459	0
M	18	4	4	4	7	1	0	0	1	0	1	0	0	0	12,019	4,218	0
F	22	10	36	3	4	1	0	1	0	0	0	1	0	0	37,745	19,718	0
F	22	8	24	8	7	1	0	0	1	0	1	1	1	1	22,867	11,872	0
F	22	20	24	14	7	0	0	0	1	0	1	1	1	1	36,84	21,951	1
F	20	2	12	4	3	0	0	0	0	0	0	1	1	1	10,17	4,5463	0
F	24	10	96	5	6	1	0	0	0	0	0	1	1	1	12,303	6,2798	1
M	28	4	24	4	4	0	0	0	0	0	0	0	0	0	19,801	16,643	0
M	41	4	8	25	3	1	0	0	0	0	0	1	1	1	9,0955	4,3225	0
M	22	8	5	1	7	1	0	0	0	0	0	0	0	0	95,433	41,914	0