1. **Title:** "*To investigate the role of Aquaporin, and blood brain barrier dysfunction and neuroinflammation markers by examining blood and CSF of patients with Idiopathic Intracranial Hypertension*"

2. **Introduction:**

Idiopathic intracranial hypertension (IIH) is a neurological condition characterized by elevated cerebrospinal fluid (CSF) pressure with normal neurological imaging and without any intracranial pathology or secondary causes of intracranial hypertension, for which factor for the cause can't be detected. The reported incidence of IIH is 0·9 to 2·2 per 100 000 in the general population, increasing to more than 19 per 100 000 in studies restricted to young, overweight women. This incidence is expected to increase additionally with more prevalence of obesity (1). Pretentious individuals are subjected to chronic disabling headaches along with pronounced visual loss, which can be drastic and may be permanent in up to 25% of the affected individuals (2).

The homeostasis of the central nervous system (CNS) microenvironment requires blood-brain barrier (BBB) integrity. The quiescent BBB is a physical and functional structure organized by brain endothelial cells (ECs) that are in contact with various CNS cell types, such as pericytes and astrocytes. The integrity and low permeability of the BBB are essential for proper neuronal function in the CNS (3).

BBB dysfunction contributes to the initiation of many neuroinflammatory diseases, including demyelinating diseases, brain tumors, and infections. Although reported evidence has shown that BBB dysfunction is associated with progressive neuroinflammation, there is still no effective or reliable marker for clinical diagnosis or to guide treatment due to limited knowledge of the molecular mechanism underlying BBB breakdown (3). The cause of BBB leakage in IIH remains unclear. Monitoring BBB integrity facilitates predicative disease prognosis and guides treatment (5).

Aquaporins might provide a link to increased intracranial pressure as they crucially contribute to water transport in brain tissue (6). Aquaporin 1 is widely distributed in the human brain and is associated with water secretion into the subarachnoid space. Aquaporin 1 was also shown to participate in the regulation of weight. According to an animal study, AQP1 mRNA and protein levels were elevated in the obese compared to lean animals (7). AQP1 is expressed in the choroid plexus and participates in forming cerebrospinal fluid. AQP4, found in astrocyte foot processes, glia limitans, and ependyma, facilitates water movement into and out of the brain, accelerates astrocyte migration, and alters neuronal activity (8). Since aquaporins have a strong relation to obesity, so it can be linked to IIH.

The Renin-angiotensin system (RAS) is an endocrine system widely known for its physiological roles in electrolyte homeostasis, body fluid volume regulation, and cardiovascular control in the peripheral circulation (10), and are expressed in neurons, astrocytes, oligodendrocytes, and microglia of various sections of brain (11).

Altered RAS plays a key role in numerous degenerative diseases of the brain including Parkinson disease (PD), Alzheimer's (AD), Huntington disease, Dementia, Amyotrophic

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lateral sclerosis, Multiple sclerosis, Traumatic brain injury, and Stroke crossing the BBB. Several reports suggested that both renin and AGT are co-expressed in many parts of the brain (10). Mice in which the angiotensinogen gene had been deleted show impairment in BBB function (12).

Since there are no extensive and detailed studies of all these parameters in IIH, however, the interrelationship between these parameters leading to IIH is still unclear. Hence, it becomes essential not only to explore but also to have an understanding of the interrelationship and correlation of these markers with the disease. So, the present study is designed to evaluate these markers and correlate their levels with disease severity and to evaluate each of its role in the pathogenesis of IIH as these maybe some of the contributing factors for IIH that needs to be explored.

Research hypothesis: IIH is a disease of unknown aetiology. The comorbidities such as Obesity and hormonal changes in the body might play a major role in causing IIH resulting in neuroinflammation and blood brain barrier dysfunction due to which there are altered levels of some proteins like increase AQP1 which helps in CSF production and AQP4 which helps in maintaining water movements in the brain. Along with this, the RAS system which also aids in CSF production, body fluid volume regulation, and electrolyte homeostasis is downregulated. So, the present study is designed to correlate the levels of these proteins and to evaluate each of its roles in the pathogenesis of IIH.

3. Objectives:

i. To evaluate Aquaporin (AQP1, AQP4) in blood and CSF of patients with IIH.
ii. To evaluate the markers of blood brain barrier dysfunction and neuroinflammation in patients of IIH like Renin and Angiotensin in blood and CSF of patients with IIH.
iii. To corelate the levels of these markers with disease severity.

4. Material and Methods:

Type of study: Hospital-based, Case-Control Study.

Study population:

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- *Cases:* IIH patients
- *Controls (AC)*: Age and Sex matched individuals undergoing spinal anaesthesia for non-neurological elective surgery.
- *Controls (AC)*: Age and Sex matched normal healthy individuals

Sample size: 40 in each arm

Sample size justification: The sample size was calculated by using the site https://www.openepi.com/SampleSize/SSCC.html. The exact prevalence of IIH in India is not known. Worldwide, the annual incidence of IIH has been reported to be 0.9/100,000 persons but it is 3.5/100,000 in females within 15 to 44 years of age. So, for the purpose of sample size calculation, we took the prevalence of IIH in the general population to be 1/100000. Hence

taking into account the controls per subject =1, alpha =0.05, and beta = 0.2, we estimated the sample size as 40 patients in each arm.

Patient population: Patients of IIH

Inclusion criteria:

For cases: Newly diagnosed IIH patients with age >18years.

For controls:

For blood and CSF sample: Age and Sex matched individuals going for non-neurological elective surgery.

For Blood sample only: Age and Sex matched normal healthy individuals

Exclusion criteria:

For cases:

1- Unwillingness to provide written informed consent.
2- Pregnancy

For controls:

1- Unwillingness to provide written informed consent.

PICOT FORMAT

Population: Patients of IIH

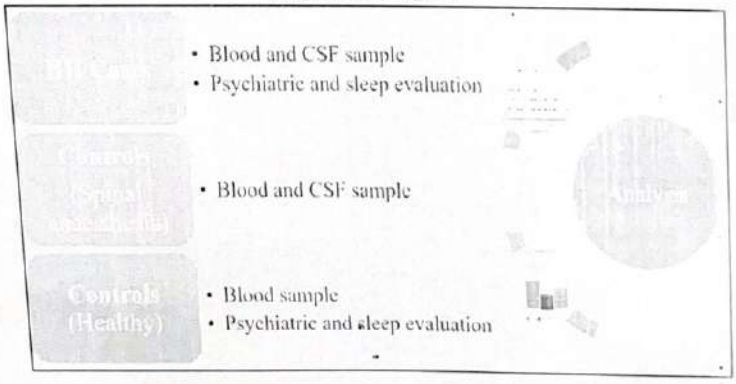
Intervention/ Exposure: Aquaporin (AQP1, AQP4), Renin and Angiotensin.

Comparison: Age and Sex matched healthy individuals undergoing spinal anaesthesia for non-neurological elective surgery.

Outcome: Altered levels of Aquaporin1, Aquaporin4, Renin and Angiotensin in CSF and blood of patients with IIH.

Time Duration: 2 years.

Study Flow Diagram



PROJECT IMPLEMENTATION

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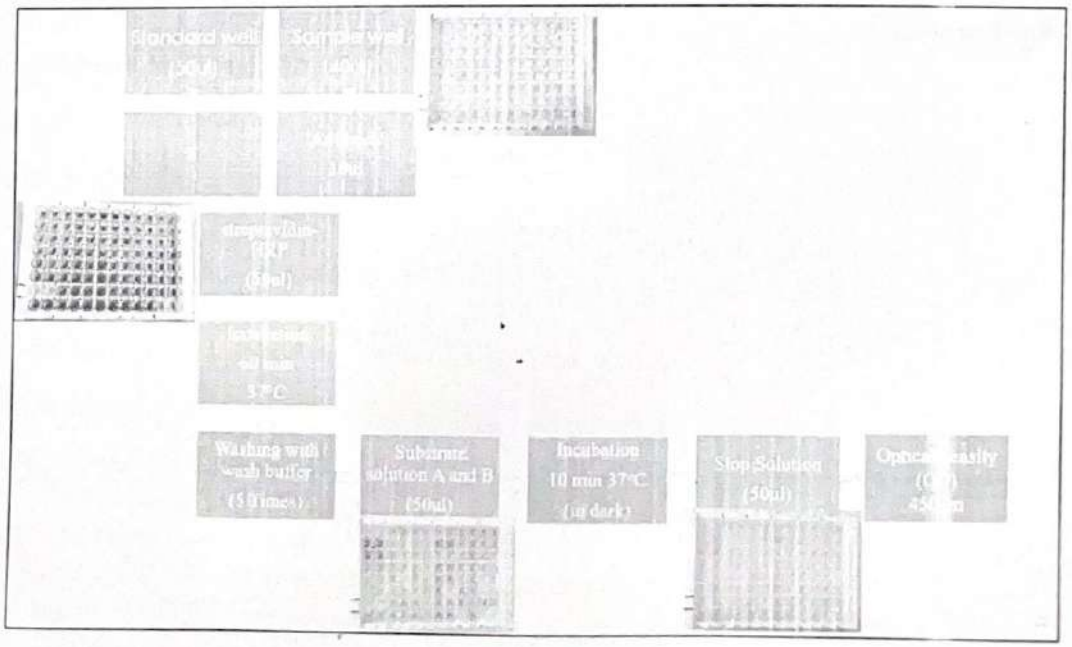
2- After obtaining informed consent, detailed history and examination was carried out in all the patients as per proforma.
3- All patients were subjected to a detailed neurological examination.
4- After being a confirmed case of IIH, 3 ml blood and CSF sample was withdrawn.
5- Blood was left for clotting for 30 minutes; the samples were centrifuged at 2200-2500RPM for 15 minutes.
6- The serum and CSF samples were then transferred to a separate biochemical tube and stored at -80°C until the day of evaluation.
7- Serum and CSF Aquaporin (AQP1, AQP4), Renin and Angiotensin levels were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit from Bioassay Technology Laboratories (BT), as per the manufacturer's instructions.
8- Samples were processed as soon as the requirement for processing a single kit will be fulfilled.
9- Serum and CSF marker levels were measured in the ELISA reader (TeCan-Spectrophotometer) at 450 nm.

Flow diagram for ELISA:



5. Results:

Baseline data

S. No	Measure	IIH Cases (N=60)	Control (SA) (N=40)	Control (Healthy) (N=40)	P-value
1.	Age (years)*	36.62 ± 9.47	40 ± 12	32 ± 7.5	0.061
2.	Male/Female	7/53	17/23	4/36	
3.	Weight (Kg)*	73.13 ± 13.18	63.75 ± 4.79	57.83 ± 8.51	**<0.001**
4.	Height (cm)*	148.8 ± 47.87	-	161.41 ± 10.19	0.666
5.	BMI (Kg/m^2)* • Underweight (<18.5)ˆ • Healthy weight (18.5-24.9)ˆ • Overweight (25-29.9)ˆ • Obese (>30)ˆ	27.81 ± 5.24 0 21 (35) 20 (33.33) 19 (31.67)	-	22.3 ± 3.38 4 (10) 31 (77.5) 4 (10) 1 (2.5)	**<0.001**

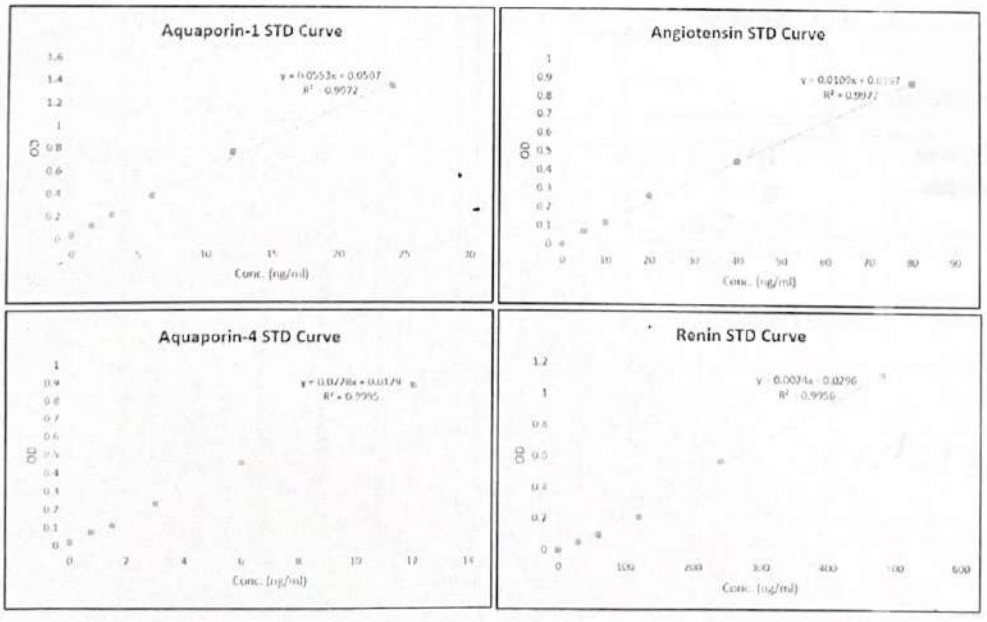
* Mean ± SD

ˆn(%)

Experimental data:

ELISA done in triplicate for each sample for the marker's aquaporin (aqp1, aqp4), renin, angiotensin in serum and CSF samples of cases and controls.

ELISA Standard curves for the markers:



Results:

Tables for Serum and CSF values of the markers in IIH patients (IIH), Anaesthesia controls (AC) and Healthy controls (HC).

Aquaporin-1

Type of sample	IIH ng/ml Median (IQR)	AC ng/ml Median (IQR)	HC ng/ml Median (IQR)	P-value
Serum	8.01 (5.52,11.69)	7.14 (4.4,11.94)	5.3 (4.35,7.98)	0.214
CSF	10.08 (7.52,12.5)	9.72 (6.96,11.59)	-	0.507

Aquaporin-4

Type of sample	IIH ng/ml Median (IQR)	AC ng/ml Median (IQR)	HC ng/ml Median (IQR)	P-value
Serum	2.65 (2.09,3.7)	3.07 (1.65,7.15)	2.6 (1.7,4.4)	0.777
CSF	3.7 (2.46,4.46)	4.34 (3.39,5.62)	-	**0.016**

Renin

Type of sample	IIH ng/ml Median (IQR)	AC ng/ml Median (IQR)	HC ng/ml Median (IQR)	P-value
Serum	80.76 (58.86,123.71)	79.86 (59.1,156.13)	57.75 (43.19,102.1)	0.09
CSF	164.47 (141.43,180.03)	211.74 (137.86,337.34)	-	**0.008**

Angiotensin

Type of sample	IIH ng/ml Median (IQR)	AC ng/ml Median (IQR)	HC ng/ml Median (IQR)	P-value
Serum	17.62 (14.54, 27.95)	15.26 (10.23,39.33)	14.91 (11.07,33.23)	0.823
CSF	29.34 (19.21,36.95)	24.83 (17.75,31.26)	-	0.112

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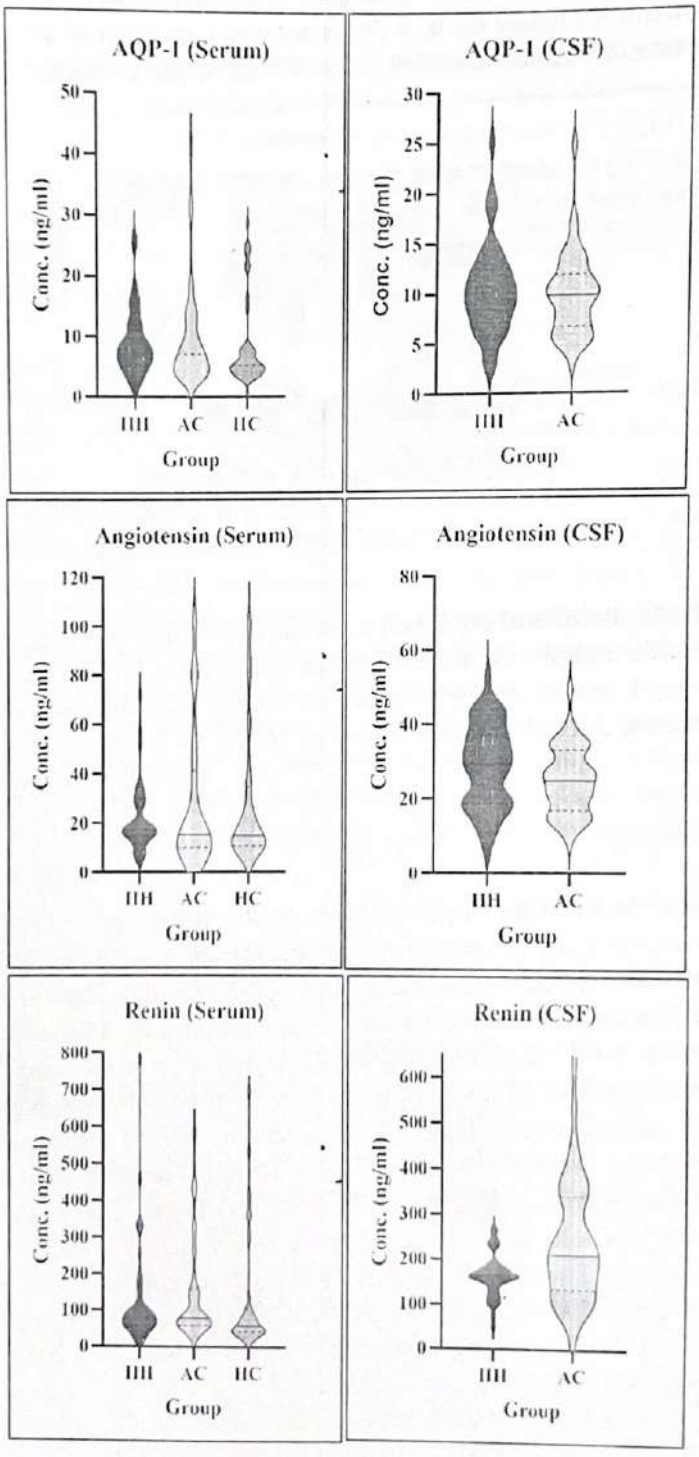
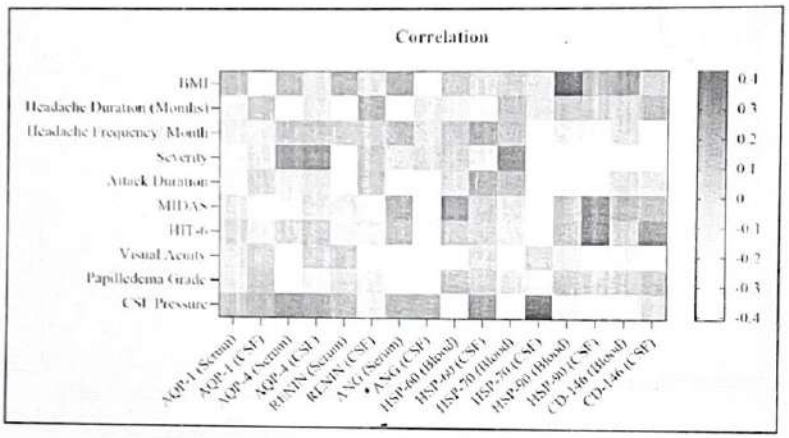
Figures showing Violin plot graphs for serum and CSF concentration of markers (Aquaporin-1, Aquaporin-4, Renin and Angiotensin) between IIH patients (IIH), Anaesthesia controls (AC) and Healthy controls (HC).



Heat map for the correlation of markers with disease parameters and severity of disease:

As we go from zero towards the darker shade, it shows positive correlation of the marker with the respective parameter, the darker the more positive and significant correlation. And as we go from zero towards the lighter shade, it shows negative correlation of the marker with the respective parameter, the lighter the more negative and significant correlation.



6. **Statistical Analysis:** A structured excel sheet was made for entering all the data. SPSS version-27 was used for all the analysis. The normality of data was checked using Kolmogorov Smirnov test. Mann Whitney U test was used to compare two variables. For categorial data, Chi square test was used. Spearman correlation test was used for correlations.

7. **Discussion:**

This study explored the levels of Aquaporins (AQP1, AQP4), Renin, and Angiotensin in blood and CSF of IIH patients and their correlation with disease severity. AQP4 levels in CSF were significantly lower in IIH patients compared to controls, suggesting impaired water regulation within the brain. This may contribute to elevated intracranial pressure, a hallmark of IIH, and indicate blood-brain barrier dysfunction, as AQP4 is crucial in maintaining brain water homeostasis. CSF Renin levels were significantly higher in IIH patients, implying central RAS activation, which may exacerbate fluid retention and contribute to increased intracranial pressure. Although Angiotensin levels were also elevated in IIH patients, the difference was not statistically significant. Higher levels of CSF AQP4, Renin, and Angiotensin were positively correlated with disease severity, indicating their potential as biomarkers for IIH progression. This suggests a role for these markers in assessing disease severity and guiding treatment. Hence this study highlights the potential of CSF AQP4 and Renin as biomarkers in IIH, with possible therapeutic implications targeting the RAS. However, the small sample size and observational nature of the study limit generalizability, warranting further research with larger cohorts. Overall, this study demonstrates altered levels of AQP4 and Renin in the CSF

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of IIH patients, correlating with disease severity, underscoring their potential role in the pathogenesis and management of IIH.

8. Impact of the research in the advancement of knowledge or benefit to mankind:

This research has significant implications for the understanding and management of IIH, a condition that can lead to severe headaches, vision problems, and even permanent vision loss if untreated. The study focuses on evaluating key biomarkers such as AQP1, AQP4, Renin, and Angiotensin in the blood and CSF of patients with IIH. By identifying and correlating the levels of these markers with disease severity, this research contributes to a deeper understanding of the pathophysiological mechanisms underlying IIH.

Advancement of Knowledge:

1. *Identification of Biomarkers:* The study potentially identifies AQP1, AQP4, Renin, and Angiotensin as biomarkers for IIH, providing new insights into the disease's molecular and biochemical basis. Understanding these markers' roles in IIH could lead to the development of novel diagnostic and therapeutic strategies.
2. *Blood-Brain Barrier Dysfunction and Neuroinflammation:* By evaluating these markers, the research contributes to the understanding of blood-brain barrier dysfunction and neuroinflammation in IIH. This knowledge can be instrumental in developing targeted therapies aimed at reducing neuroinflammation and stabilizing the blood-brain barrier, which may alleviate symptoms and prevent disease progression.
3. *Comparative Analysis:* The comparison between IIH patients and age- and sex-matched controls, including those undergoing spinal anaesthesia and healthy individuals, allows for a more nuanced understanding of how these markers differ in pathological versus normal states. This comparative approach strengthens the validity of the findings and their potential application in clinical settings.

Benefit to Mankind:

1. *Improved Diagnosis and Monitoring:* The identification of reliable biomarkers for IIH can lead to earlier and more accurate diagnosis, which is crucial for preventing irreversible complications such as vision loss. Moreover, these markers can be used to monitor disease progression and treatment response, allowing for more personalized and effective patient management.
2. *Therapeutic Development:* Understanding the role of Aquaporins, Renin, and Angiotensin in IIH can pave the way for the development of new therapeutic approaches. For instance, if these markers are found to play a significant role in disease progression, drugs targeting these molecules could be developed to treat or even prevent IIH.
3. *Public Health Implications:* As IIH is a rare but serious condition, this research can raise awareness among clinicians and the general public about the importance of early detection and management. The findings could also inform public health strategies aimed at identifying at-risk populations and implementing preventive measures.

In summary, this research represents a critical step forward in the understanding and management of IIH. It not only enhances our knowledge of the disease but also holds the

potential to improve patient outcomes and quality of life through better diagnostic and therapeutic tools.

9. Literature references

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Type of study: Hospital-based, Case-Control Study.

Study population:

- *Cases:* IIH patients
- *Controls (AC)*: Age and Sex matched individuals undergoing spinal anaesthesia for non-neurological elective surgery.
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Sample size: 40 in each arm

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PICOT FORMAT

Population: Patients of IIH

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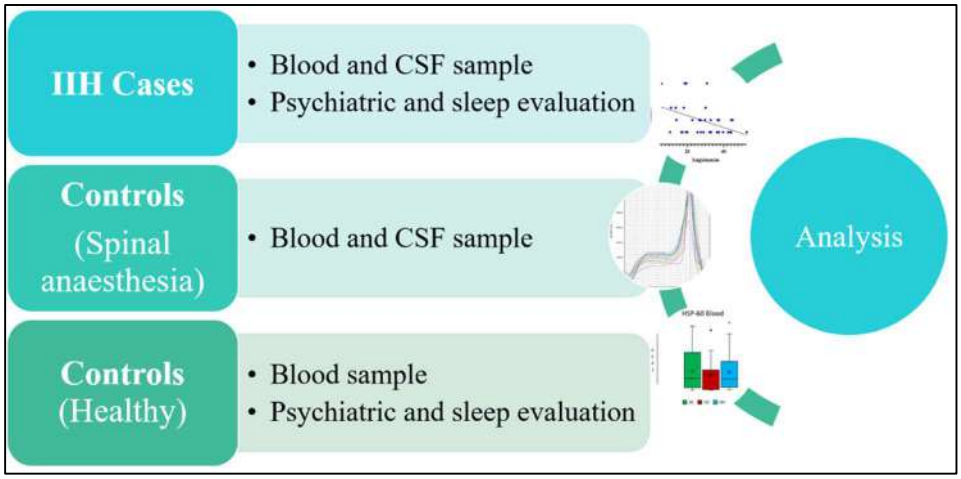
Outcome: Altered levels of Aquaporin1, Aquaporin4, Renin and Angiotensin in CSF and blood of patients with IIH.

Time Duration: 2 years.

Study Flow Diagram



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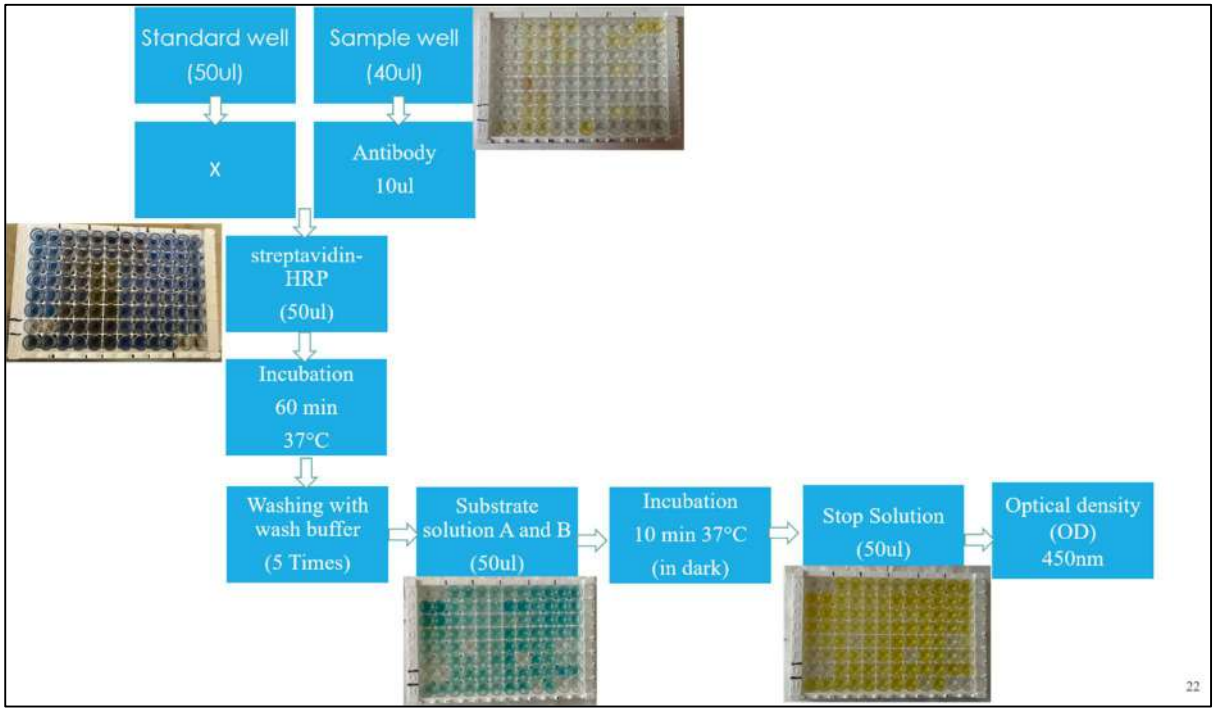

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* Mean ± SD
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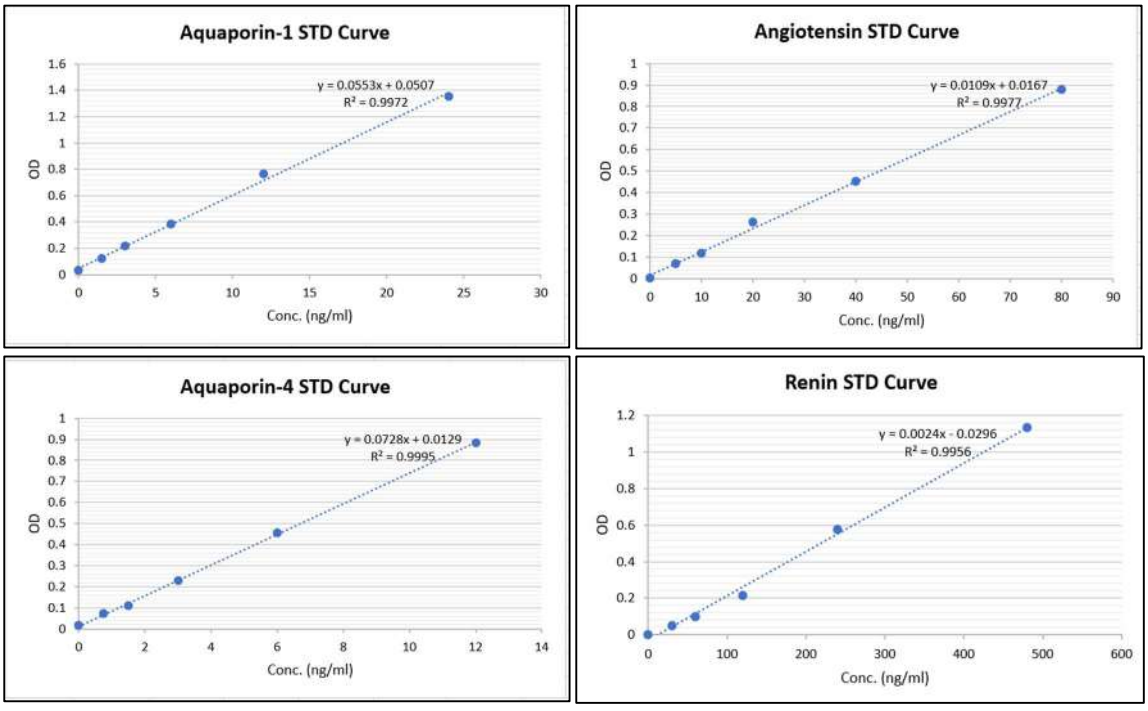

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CSF	10.08 (7.52,12.5)	9.72 (6.96,11.59)	-	0.507

Aquaporin-4

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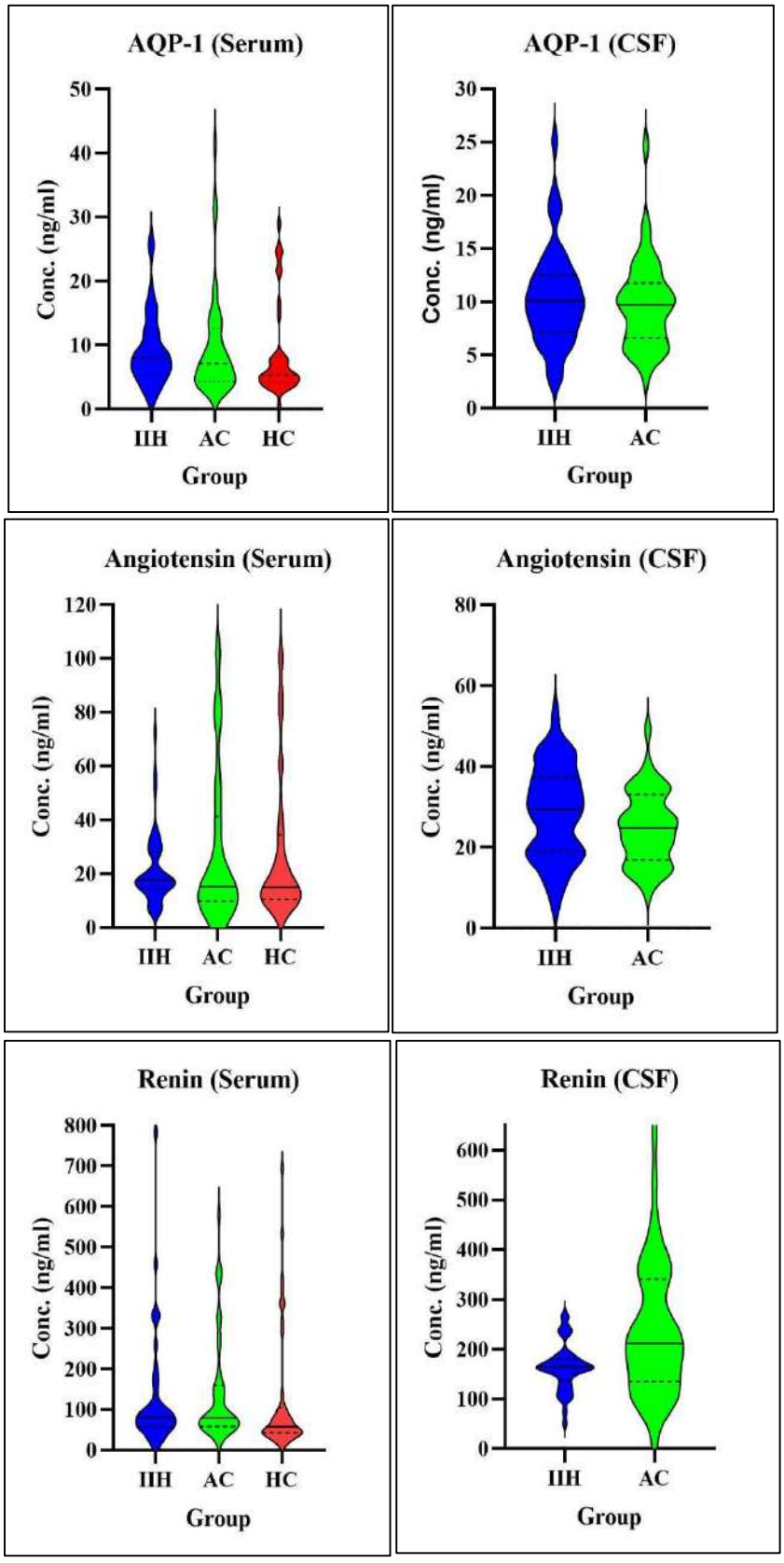
Type of sample	**IIH** ng/ml Median (IQR)	**AC** ng/ml Median (IQR)	**HC** ng/ml Median (IQR)	**P-value**
Serum	80.76 (58.86,123.71)	79.86 (59.1,156.13)	57.75 (43.19,102.1)	0.09
CSF	164.47 (141.43,180.03)	211.74 (137.86,337.34)	-	**0.008**

Angiotensin

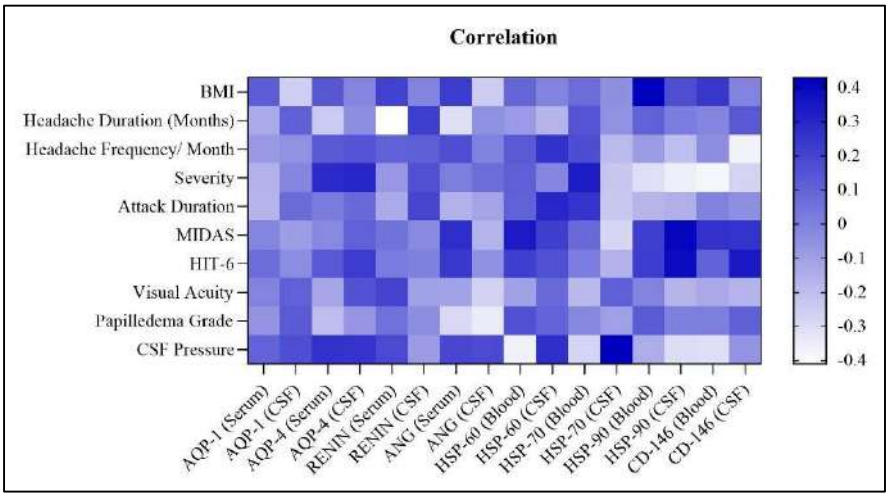
Type of sample	**IIH** ng/ml Median (IQR)	**AC** ng/ml Median (IQR)	**HC** ng/ml Median (IQR)	**P-value**
Serum	17.62 (14.54, 27.95)	15.26 (10.23,39.33)	14.91 (11.07,33.23)	0.823
CSF	29.34 (19.21,36.95)	24.83 (17.75,31.26)	-	0.112

Figures showing Violin plot graphs for serum and CSF concentration of markers (Aquaporin-1, Aquaporin-4, Renin and Angiotensin) between IIH patients (IIH), Anaesthesia controls (AC) and Healthy controls (HC).



Heat map for the correlation of markers with disease parameters and severity of disease:

As we go from zero towards the darker shade, it shows positive correlation of the marker with the respective parameter, the darker the more positive and significant correlation. And as we go from zero towards the lighter shade, it shows negative correlation of the marker with the respective parameter, the lighter the more negative and significant correlation.



6. **Statistical Analysis:** A structured excel sheet was made for entering all the data. SPSS version-27 was used for all the analysis. The normality of data was checked using Kolmogorov Smirnov test. Mann Whitney U test was used to compare two variables. For categorial data, Chi square test was used. Spearman correlation test was used for correlations.

7. **Discussion:**

This study explored the levels of Aquaporins (AQP1, AQP4), Renin, and Angiotensin in blood and CSF of IIH patients and their correlation with disease severity. AQP4 levels in CSF were significantly lower in IIH patients compared to controls, suggesting impaired water regulation within the brain. This may contribute to elevated intracranial pressure, a hallmark of IIH, and indicate blood-brain barrier dysfunction, as AQP4 is crucial in maintaining brain water homeostasis. CSF Renin levels were significantly higher in IIH patients, implying central RAS activation, which may exacerbate fluid retention and contribute to increased intracranial pressure. Although Angiotensin levels were also elevated in IIH patients, the difference was not statistically significant. Higher levels of CSF AQP4, Renin, and Angiotensin were positively correlated with disease severity, indicating their potential as biomarkers for IIH progression. This suggests a role for these markers in assessing disease severity and guiding treatment. Hence this study highlights the potential of CSF AQP4 and Renin as biomarkers in IIH, with possible therapeutic implications targeting the RAS. However, the small sample size and observational nature of the study limit generalizability, warranting further research with larger cohorts. Overall, this study demonstrates altered levels of AQP4 and Renin in the CSF

of IIH patients, correlating with disease severity, underscoring their potential role in the pathogenesis and management of IIH.

8. Impact of the research in the advancement of knowledge or benefit to mankind:

This research has significant implications for the understanding and management of IIH, a condition that can lead to severe headaches, vision problems, and even permanent vision loss if untreated. The study focuses on evaluating key biomarkers such as AQP1, AQP4, Renin, and Angiotensin in the blood and CSF of patients with IIH. By identifying and correlating the levels of these markers with disease severity, this research contributes to a deeper understanding of the pathophysiological mechanisms underlying IIH.

Advancement of Knowledge:

1. *Identification of Biomarkers:* The study potentially identifies AQP1, AQP4, Renin, and Angiotensin as biomarkers for IIH, providing new insights into the disease's molecular and biochemical basis. Understanding these markers' roles in IIH could lead to the development of novel diagnostic and therapeutic strategies.
2. *Blood-Brain Barrier Dysfunction and Neuroinflammation:* By evaluating these markers, the research contributes to the understanding of blood-brain barrier dysfunction and neuroinflammation in IIH. This knowledge can be instrumental in developing targeted therapies aimed at reducing neuroinflammation and stabilizing the blood-brain barrier, which may alleviate symptoms and prevent disease progression.
3. *Comparative Analysis:* The comparison between IIH patients and age- and sex-matched controls, including those undergoing spinal anaesthesia and healthy individuals, allows for a more nuanced understanding of how these markers differ in pathological versus normal states. This comparative approach strengthens the validity of the findings and their potential application in clinical settings.

Benefit to Mankind:

1. *Improved Diagnosis and Monitoring:* The identification of reliable biomarkers for IIH can lead to earlier and more accurate diagnosis, which is crucial for preventing irreversible complications such as vision loss. Moreover, these markers can be used to monitor disease progression and treatment response, allowing for more personalized and effective patient management.
2. *Therapeutic Development:* Understanding the role of Aquaporins, Renin, and Angiotensin in IIH can pave the way for the development of new therapeutic approaches. For instance, if these markers are found to play a significant role in disease progression, drugs targeting these molecules could be developed to treat or even prevent IIH.
3. *Public Health Implications:* As IIH is a rare but serious condition, this research can raise awareness among clinicians and the general public about the importance of early detection and management. The findings could also inform public health strategies aimed at identifying at-risk populations and implementing preventive measures.

In summary, this research represents a critical step forward in the understanding and management of IIH. It not only enhances our knowledge of the disease but also holds the

potential to improve patient outcomes and quality of life through better diagnostic and therapeutic tools.

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