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ORIGINAL ARTICLE

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Serial perioperative optic nerve sheath measurements for early diagnosis of the transurethral resection of prostate syndrome: an open label pilot study

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Abstract

Background Ultrasound imaging of optic sheath nerve diameter [ONSD] is reported to reflect changes consistent with intracranial pressure changes seen in traumatic brain injury and also in documented serum hyponatremia. We hypothesized that hyponatremia and hypervolemia seen during trans urethral resection of prostate [TURP] surgery may also have some association with different ONSD readings from the baseline perioperatively, resulting in early detection of TURP syndrome. In this prospective observational study, 50 adult male patients scheduled for TURP surgery meeting inclusion criteria were included and the ONSD measurements were serially recorded perioperatively. Patients with measurements ≥ 5.2 mm with either clinical symptoms or electrolyte changes suggested TURP syndrome were taken as true positive.

Results The sensitivity, specificity, area under the curve, positive predictive, and negative predictive value at 95%CI of ONSD for early detection of TURP syndrome was {100% [15.81 to 100.00%], 91.67% [80.02 to 97.68%], 0.96 [0.86 to 0.99%], 33.33% [4.33 to 77.72%], 100% [91.96 to 100.00%]} with a diagnostic accuracy of 95.83%. In univariate logistic regressions, the duration of surgery had a positive association with TURP syndrome [odd ratio 1.066, β coefficient 0.064, $p = 0.015$]. In multivariate logistic regression, we could not validate the association between these factors and TURP syndrome [$p > 0.050$].

Conclusions The ONSD measurements have good diagnostic accuracy for detecting TURP syndrome, but we advocate more multi-centric studies with large sample sizes to validate this association in the multivariate regression model.

Keywords Ultrasonography, Trans urethral resection of the prostate, Intracranial pressure, Optic nerve, Hyponatremia, Cautery

Background

Transurethral resection of prostrate [TURP] syndrome may occur during endoscopic surgery for prostatomegaly with a varying reported incidence between 0.18 to 20% with varying mortality of 0.2–0.8 (Nakihara et al. 2014; Hazakika 2020). Hypervolemia, hyponatremia, and hypo-osmolality develop in the patient due to the absorption of irrigation fluid, which can increase intracerebral pressure [ICP] and lead to clinical symptoms of TURP syndrome.

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The lithotomy position is known to cause increased pressure on subarachnoid space which further translocate a portion of cerebrospinal fluid [CSF] volume present in the spinal canal to the ventricular ducts in the brain. As the optic nerve is in direct continuation with the forebrain, the increase in ICP gets transmitted to the sheath surrounding the optic nerve. Sonographic or magnetic resonance assessment of the diameter of this sheath around the optic nerve, gives a value correlating with the elevated ICP. A significant correlation exists in the literature between elevated ICP with increased ONSD. Therefore, our research question was "Can TURP syndrome be diagnosed early by serial intraoperative ONSD measurement"? This question became imperative because the clinical symptoms of TURP syndrome are nonspecific and the estimation of serum electrolytes takes a considerable delay.

Methods

This clinical research study was approved by the institutional ethical committee and was registered with CTRI/2020/10/028448 and conforms to the STROBE cohort study checklist.

Sahay et al. (2018) conducted a prospective study and observed the mean difference between baseline ONSD [0.44 + 0.03 cm] and ONSD after pneumoperitoneum [0.47 + 0.04 cm] to be 0.03 cm. With confidential intervals of 95 and a power of 80%, the sample size came out to be 44 patients for the present study.

We considered 55 patients for the study but 5 patients had to be excluded [2 had mean ONSD ≥ 5 mm at baseline, and 3 refused to cooperate for measurements]. Therefore, the finalized prospective study cohort comprised 50 male patients between the age of 40 to 80 years. As the period of the study was during the Covid pandemic when routine surgeries were deferred hence, we took a convenience sample of those patients who were scheduled for TURP surgery in our institution over one year period from October 2020 to September 2021. Patients with pre-existing glaucoma, cerebral neoplasm, orbital injury, ventriculoperitoneal [VP] shunt, and post-neurosurgery status were excluded from the study. Likewise, the patients with baseline ONSD < 3 mm or ≥ 5 mm were also excluded from the study cohort.

After obtaining valid informed consent, the patients were explained the procedure and they were advised to keep their eyes closed during the procedure. A high frequency 7.5–15 MHz linear array transducer [Sonosite] was used for ONSD measurement under B mode. The ultrasonic gel was applied over the eyelid, and the examination in the sagittal plane was done followed by an examination in the transverse plane, by rotating the transducer to 90 degrees from the initial plane of examination. The optic nerve sheath was identified and the image was frozen, thereafter measurements were taken 3 mm behind the optic disc. This served as a baseline measurement of ONSD. Subsequent measurement of ONSD was done on other eyeball and the mean value was obtained. Thereafter, ONSD measurements were taken at the set time points as per our study protocol by the principal investigator in our study and the hemodynamic measurements were recorded by the rest of the investigators.

The anesthetic and surgical techniques were performed at the discretion of the attending anesthetist and the urologist respectively. After securing an intravenous line with 18 G cannula, normal saline was used as the fluid intraoperatively. Regional anesthesia was given in all the patients in the form of the single shot spinal block with 3 ml of 0.5% bupivacaine intrathecally.

1. The mean ONSD was recorded at baseline, at the onset of lithotomy position, and subsequently at every hour of surgery until its end.
2. Hemodynamic parameters were noted down every 5 min as per the ASA standard monitoring [MAP, HR, and SpO2].
3. Any occurrence of restlessness, mydriasis, irritability, hypothermia, and confusion was recorded.
4. The total duration of surgery, volume of irrigation fluid used, the height of the irrigation fluid bag from the operation table, and volume and type of intravenous fluids administered were also recorded.
5. A blood sample for postoperative serum electrolytes assessment was collected at the end of surgery.
6. During the course of the study, we came across a non-invasive model for calculating predicted ICP in accordance with Wang et al. (2017) who calculated it using an equation [Predicted ICP = − 111.92 + 77.36 × ONSD]. This equation was reported to have a good correlation with measured ONSD in modeling and test groups [$p \leq 0.001$]. We too derived predicted ICP in our study based on this equation.

A patient was designated to be a true positive case of TURP syndrome only if he had a mean ONSD ≥ 5.2 mm along with either clinical symptoms of TURP syndrome or documented hyponatremia [Na + < 135 mmol/l] and hypo osmolality.

The data entry was done in a Microsoft Excel spreadsheet and the analysis was done with the Statistical package for social sciences [SPSS] software, IBM manufactures, Chicago, USA, ver. 21.0. The presentation of categorical variables was done in the form of numbers and percentages. On the other hand, the quantitative

data were presented as mean + SD and as median with 25th and 75th percentiles (interquartile range). Results were analyzed using an independent *t* test. The sensitivity, specificity, positive predictive value, and negative predictive value of ONSD were calculated for the early detection of TURP syndrome. Univariate, stratified, and multivariate logistic regression tests were applied to the significantly associated factors for the TURP syndrome to remove any bias. A value of $p < 0.050$ was considered statistically significant.

Results

The mean + SD age of the study cohort was 67.98 ± 8.5 years, whereas the mean + SD weight of patients was 66.12 ± 6.16 kg. The mean body mass index [BMI] for the patients in our study came out to be 22.6 ± 3.03 kg/m^2.The patients who developed TURP syndrome had higher mean BMI [27.5 ± 1.29 kg/m^2] [$p < 0.001$] (Table 1).

Five patients had controlled hypertension [10.00%], 2 patients had coronary artery disease [4.00%] and diabetes mellitus, and 1 patient [2%] was of asthma but none of these patients developed symptoms of TURP syndrome in our study.

The mean ONSD was 4.27 ± 0.4 mm at baseline. It increased significantly after lithotomy to 4.33 ± 0.38 mm. Subsequently, it became 4.7 ± 0.57 mm and 6.35 [in 1 patient at 2 h] intraoperatively. It was 4.74 ± 0.65 mm in the postoperative period [$p < 0.001$] (Table 2).

The mean derived ICP by wang's mathematical expression (Sahay et al. 2018) in mm H2O at baseline was 216.86 ± 30.59. After the lithotomy position, it increased to 220.68 ± 29.42 [$p < 0.001$]. It kept on increasing in the operative period and was 248.73 ± 44.26 and 344 ± 0 at 1 and 2 h respectively and thereafter it became 251.3 ± 42.65 in the postoperative period. The derived mean ICP was high without any statistical significance in symptomatic patients [$p > 0.050$] (Table 2).

The preoperative and postoperative mean values of sodium were 138.24 ± 3.71 and 139.17 ± 3.63 mmol/l in the asymptomatic patients, whereas it was 135.25 ± 2.75 and 125.88 ± 19.33 mmol/l in the symptomatic patients [$p > 0.050$]. The preoperative serum potassium was

Table 1 Association of demographic characteristics and other factors with TURP syndrome

Demographic characteristics and other factors	Symptoms absent ($n = 46$)	Symptom's present ($n = 4$)	Total	*P* value
Age (years)				
Median (25th–75th percentile)	69(61–73)	71.5(69.25–75.5)	69.5(61.75–73)	
Mean ± SD	67.52 ± 8.52	73.25 ± 6.95	67.98 ± 8.49	0.199*
Body mass index (kg/m^2)				
< 18.5{underweight}	2 (4.35%)	0 (0%)	2 (4%)	
18.5–24.99{normal BMI}	31 (67.39%)	0 (0%)	31 (62%)	
25–29.99{overweight}	13 (28.26%)	4 (100%)	17 (34%)	
Mean ± SD	22.18 ± 2.75	27.5 ± 1.29	22.6 ± 3.03	0.0004*
Duration of surgery in min	66.3 ± 22.15	111.25 ± 30.1	69.9 ± 25.64	0.0004
Height of irrigation fluid (cm)	60.7 ± 2	61.25 ± 1.5	60.74 ± 1.96	0.592
Irrigation fluid volume in lit	19.78 ± 8.84	31 ± 10.68	20.68 ± 9.39	0.02
IV fluids infused in ml	670.65 ± 230.36	950 ± 404.15	693 ± 254.15	0.034
Prostate tissue in mg resected	46.7 ± 24.71	58.75 ± 16.01	47.66 ± 24.23	0.345
Frusemide used mg	23.33 ± 15.28	40 ± 30.28	32.86 ± 24.81	0.429

Table 2 Descriptive statistics of ONSD measurement (mm) and derived ICP (mm H2O) of study subjects

Time of measurement	ONSD Mean ± SD (range)	ONSD Median (25th–75th percentile)	Derived ICP Mean ± SD (Range)	Derived ICP Median (25th–75th percentile)	*P* value w.r.t pre-operative
Baseline	4.27 ± 0.4 (3.55–4.8)	4.4(3.9–4.59)	216.86 ± 30.59 (158–259)	220(189–242.25)	–
Lithotomy	4.33 ± 0.38 (3.6–4.85)	4.4(3.95–4.6)	220.68 ± 29.42 (166–259)	228(190–243)	< 0.0001
1 h	4.7 ± 0.57 (3.85–6.75)	4.65(4.35–4.975)	248.73 ± 44.26 (182–406)	247(222–267)	< 0.0001
2 h ($n = 1$)	6.35 ± 0 (6.35–6.35)	6.35(6.35–6.35)	344 ± 0 (344–344)	344	–
Postoperative	4.74 ± 0.65 (3.8–7.9)	4.72(4.35–4.988)	251.3 ± 42.65 (182–419)	251(220–272.25)	< 0.0001

4.34 ± 0.43 mmol/l whereas postoperatively it was 4.32 ± 0.63 mmol/l [$p = 0.892$].

The mean duration of surgery was 66.3 ± 22.15 min in asymptomatic patients and 111.25 ± 30.1 min in symptomatic patients [$p < 0.0004$]. The mean height of irrigation fluid used during surgery was 60.74 ± 1.96 cm [$p = 0.592$]. The mean volume of irrigation fluid used was 19.78 ± 8.84 L in the asymptomatic patients and 31 ± 10.68 L in symptomatic patients [$p = 0.029$]. The mean value of IV fluid used was 670 ± 230.36 ml in asymptomatic patients and 950 ± 404.15 ml in symptomatic patients [$p = 0.034$]. The mean weight of the prostate tissue resected was 46.7 ± 24.71 g in asymptomatic patients and 58.75 ± 16.01 g in symptomatic patients [$p = 0.345$]. The mean value of the intraoperative frusemide dose was 32.86 ± 24.81 mg. It was 23.33 ± 15.28 mg in asymptomatic and 40 ± 30.28 mg in symptomatic patients [$p = 0.429$].

Bipolar cautery was used in 16 patients and monopolar cautery was used in 34 patients. Mono-polar cautery was used in all 4 patients who developed symptoms without any statistical significance [$p = 0.292$] (Table 1).

We did not find any statistical significance in the percentage fall in blood pressure with respect to baseline values in patients with or without TURP syndrome (Fig. 1).

Postoperatively 1 patient [2.00%] had dry mouth, 2 patients [4.00%] had bradycardia, 1 patient [2.00%] had hypotension, 2 patients [4.00%] suffered from hypothermia and mental confusion, 1 patient [2.00%] had postoperative nausea, vomiting and shivering, and 2 patients required intensive care at the ICU.

The sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], AUC with 95% confidence interval [CI] was [100% (15.81 to 100.00%); 91.67% (80.02 to 97.68); 33.33% (4.33 to 77.72%); 100% (91.96 to 100.00%); AUC 0.96 (0.86 to 0.99)] with a diagnostic accuracy of 95.83% for the cut-off value of ONSD ≥ 5.2 mm for diagnosing TURP syndrome (Table 3).

We did a univariate logistic regression and observed that the duration of surgery had a positive association with TURP syndrome with an odd ratio of 1.066 and β coefficient of 0.064 [$p = 0.015$]. The volume of irrigation fluid used and BMI > 25 had a weak association with TURP syndrome [$p = 0.05$]. We did not get any significant correlation between ONSD measurement, predictive ICP, fluids used intra-operatively, and co-morbidities with the TURP syndrome. When we applied multivariate logistic regression to these factors, we did not find any association between these factors and a predictive response in the form of TURP syndrome in our study (Tables 4 and 5).

Discussion

We studied the effectiveness of serial ONSD measurement as a proxy indicator of increased ICP. Jenson et al. (1991) reported that the neurological symptoms of TURP syndrome are because of elevated ICP and papilledema following fluid absorption during TURP surgery. We hypothesized that we would detect TURP syndrome early by ONSD estimations in the patients where other clinical features of TURP syndrome are inconclusive and definitive methods like CT, MRI, and direct ICP measurements are not possible.

We searched the literature and found studies that reported a significant association between increased ONSD and documented hyponatremia or elevated ICP.

Table 3 Sensitivity, specificity, positive predictive value, and negative predictive value of Ultrasonic optic nerve sheath diameter ONSD for early detection of TURP syndrome

Variables	Values
Sensitivity (95% CI)	100% (15.81 to 100.00%)
Specificity (95% CI)	91.67% (80.02 to 97.68%)
AUC (95% CI)	0.96 (0.86 to 0.99)
Positive predictive value (95% CI)	33.33% (4.33 to 77.72%)
Negative predictive value (95% CI)	100% (91.96 to 100.00%)
Diagnostic accuracy	95.83%

Wang et al. (2017) compared ONSD with post-lumbar puncture ICP. Frumin et al. (2014) correlated sonographic ONSD in traumatic brain injury patients with invasive ICP monitoring. Hanafi et al. (2019) studied the CT scans of patients with elevated ICP. Demir et al. (2018) found a significant association between documented hyponatremia (Na ≤ 135 mmol/l) and high ONSD of 5.46 ± 0.68 mm in symptomatic patients coming to the emergency room [$p < 0.001$]. As the mean ONSD of 5.2 mm to 5.6 mm was taken as a critical value for detecting elevated ICP of at least 200 mm H2O in these respective studies; thus, we also took 5.2 mean ONSD value as the cutoff value for diagnosing TURP in our patients.

The mean ONSD values of 4.27 ± 0.4 mm at baseline [mean age 67.98 years] observed in our study were comparable to the results of Bidur & Thapa (2018) and Whiteley et al. (2015) who reported mean values of 4.16 mm to 4.5 mm respectively. However, Zeiler et al. (2016) reported a lower ONSD of 3.68 mm in young volunteers [mean age 29.3 years].

Sahay et al. (2018) and Whiteley et al. (2015) have reported an increase in ONSD after trendlenburg position whereas Romagnuolo et al. (2005) did not find any

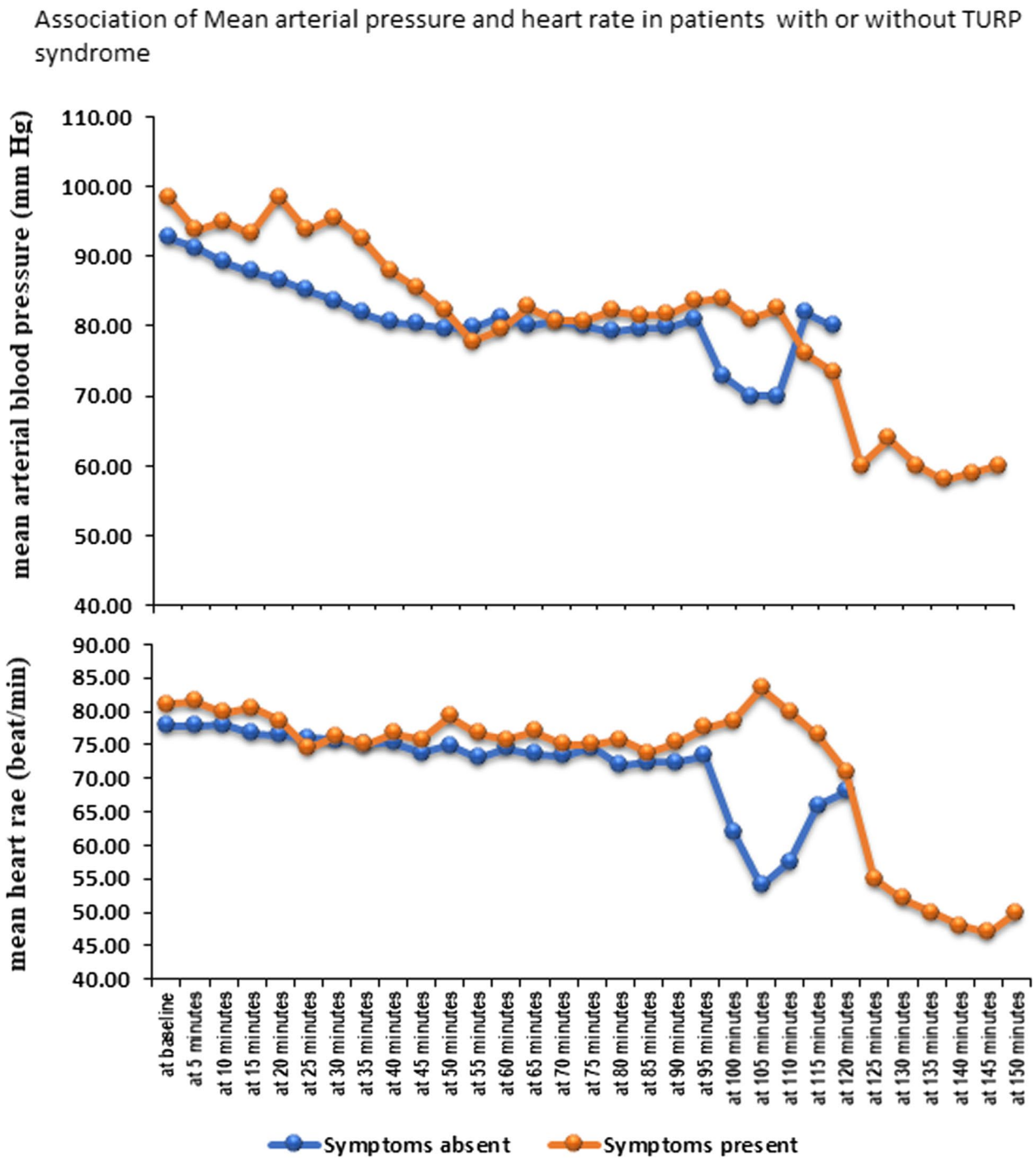

Fig. 1 Association of hemodynamic parameters with TURP syndrome

significant difference in ONSD for patients undergoing robotic prostatectomy in a steep head-down position.

In normal patients ICP is in the range of 7–15 mmHg [95–200 mm H2O] and treatment is indicated if it increases to 20–25 mmHg [270–340 mm H2O]. In the present study, the predicted ICP was in the range of 158–282 mm H2O in asymptomatic patients and 243–419 mm H2O in symptomatic patients. These differences were clinically significant; however, they were non-significant statistically in our study ($p > 0.05$).

In the present study, 6 patients had ONSD ≥ 5.2 mm; however, only 4 patients were taken as true positive as

Table 4 Univariate logistic regression to find out factors affecting TURP syndrome

Variable	Beta coefficient	Standard error	*P*	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Duration of surgery (min)	0.064	0.026	0.015	1.066	1.013	1.122
Volume of irrigation fluid (L)	0.127	0.065	0.050	1.136	1.000	1.290
ONSD measurement (mm)	4.432	2.478	0.074	84.105	0.654	10,816.671
Predictive ICP (mmH2O) at baseline	0.060	0.032	0.062	1.062	0.997	1.130
IV fluid (ml)	0.003	0.002	0.063	1.003	1.000	1.007
Body mass index (kg/m^2)						
18.5–24.99 {normal BMI}				1.000		
< 18.5 {underweight}	2.534	2.387	0.288	12.600	0.117	1355.509
25–29.9 {overweight}	3.045	1.553	0.050	21.000	1.001	440.470
Hypertension	1.398	1.176	0.234	4.048	0.404	40.564
Coronary artery disease	0.682	1.962	0.728	1.978	0.042	92.536
Diabetes mellitus	0.682	1.962	0.728	1.978	0.042	92.536
Kyphotic spine	1.215	2.363	0.607	3.370	0.033	345.813
Asthmatic	1.215	2.363	0.607	3.370	0.033	345.813

they had reported various clinical symptoms of TURP syndrome in addition to the raised serial ONSD values. In only two patients out of these 4 true positive patients, dyselectrolytemia [Na + level of 102–118 mmol/l and osmolality of 210–244 osmol/l] was documented. Thus, 2 patients who were without any clinical symptoms or electrolyte changes were taken as false positives in our study but they could have been cases of early TURP syndrome as intravenous frusemide is routinely given intraoperatively in our institution which could have modified their values to normal limits. The 2 patients with dyselectrolytemia were shifted to the ICU and underwent serial ONSD measurements. It was observed that the increase in ONSD took almost 2 days to return to the baseline value. The mean ONSD values of these symptomatic patients were 6.7 mm and 5.4 mm preoperatively, whereas it was 7.9 mm and 6.6 mm postoperatively. Thus, it further validated that the serial ONSD measurements could prove to be a good diagnostic and post-operative tool in patient management over expensive tests like CT and MRI.

In our study, the sensitivity, specificity, PPV, NPV, and AUC with 95% CI were [100%; 91.67%; 33.33%; 100%; AUC 0.96] with a diagnostic accuracy of 95.83% for the cut-off value of ONSD ≥ 5.2 mm for diagnosing TURP syndrome.

Gupta and Pachisia (2019) reported an ONSD > 6.3 mm to have a sensitivity of 77.3% [95% CI = 54.6–92] and specificity of 92.3% [95% CI = 84.0–97.1] in diagnosing elevated ICP in their study where they compared ONSD values with lumbar puncture derived ICP values [$p < 0.001$]. Demir et al. (2018) reported that the AUC was 0.870 for ONSD of 4.9 mm in symptomatic hyponatremia patients with a sensitivity of 81% and specificity of 81.9%.

To date, the onus of the diagnosis of this syndrome lies on clinical features and electrolyte estimation. Hahn

Table 5 Multivariate logistic regression to find out factors affecting TURP syndrome

Variable	Beta coefficient	Standard error	*P*	Odds ratio	Odds ratio Lower bound (95%)	Odds ratio Upper bound (95%)
Duration of surgery(minutes)	0.060	0.041	0.145	1.061	0.980	1.150
Volume of irrigation fluid (L)	− 0.049	0.097	0.615	0.952	0.787	1.152
Body mass index (kg/m^2)						
18.5–24.99 {normal BMI}				1.000		
< 18.5 {underweight}	1.643	2.370	0.488	5.170	0.050	537.945
25–29.99 {overweight}	2.203	1.487	0.139	9.050	0.491	166.916

(2006) gave 1–3 score for the clinical manifestations of TURP syndrome which includes various circulatory changes [chest pain, bradycardia, HTN, hypotension, poor urine output] and neurological symptoms [blurred vision, nausea, vomiting, confusion, tiredness, headache]. In the present study, 4 patients of TURP syndrome had mild uneasiness and shivering. Postoperatively, 2 patients developed bradycardia and 1 of them also developed hypotension. Therefore, the patients who developed overt symptoms, the Hahn severity score was ≥ 3, whereas for the 2 patients who improved with frusemide, the score was ≤ 1 in our study. Ishio et al. (2015) and Fuziwara et al. (2014) found that all patients who developed symptoms in their study had a score of ≥ 2 and those with a score of ≥ 3 had to receive additional drugs like propofol and midazolam. We also got these symptoms with a score ≥ 2 in our patients but as the signs included in this scoring are sometimes present in otherwise normal patients, like hypotension, vomiting, and hypothermia because of regional anesthesia-induced sympathetic blockade hence we feel these signs may be present in asymptomatic patients too hence they cannot be relied upon in early detection of TURP syndrome.

We observed a significant positive correlation between the duration of surgery, the volume of irrigation fluid, and intravenous fluid used in the symptomatic patients with TURP syndrome in univariate logistic regression but could not prove this association in the multivariate regression model of the study. Ishio et al. (2015) had reported a positive correlation between these factors and TURP syndrome and Bruce et al. (2021) who observed a significant risk of TURP syndrome with monopolar cautery use intra-operatively unlike our study where 4 of our patients had an insignificant association with its use.

Strength and limitations

Although the variations in ONSD values were clinically observed to be greater in symptomatic patients over asymptomatic patients but were statistically significant in the advanced statistical model of our study as the association was examined in only 50 patients during the covid pandemic period. The two patients who were taken as false positives for being asymptomatic could have been cases of early TURP syndrome as we did not cross-check them with other radiological investigations in our study. The objective assessment and the learning curve bias for the assessment of ONSD were other limitations of this study. We could not validate the wang model of calculating the predictive ICP in our patients as some of our normal preoperative patients had derived an ICP of 200 mmHg by this formula.

Conclusions

We found 95.83% diagnostic accuracy between ≥ 5.2 mm mean ONSD values and early TURP syndrome. Due to the ease of measurements and easy availability of USG in anesthesia armamentarium, we hope further multicentric studies involving a large number of patients will make these easily reproducible, non-invasive measurements a standard practice of anesthesia and surgical care in the future for patient safety.

Abbreviations

ONSD	Optic sheath nerve diameter
TURP	Trans urethral resection of the prostate
USG	Ultrasound
ICP	Intracranial pressure
mmHg	Millimetres of mercury
AUC	Area under the curve
PPV	Positive predictive value
CI	Confidence interval
NPV	Negative predictive value
BMI	Body mass index
CSF	Cerebral spinal fluid

Supplementary Information

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Additional file 1. Stobe checklist.

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Not applicable.

Authors' contributions

BC conducted the literature review and did data collection and analysis. PR performed all the cases as surgeon and helped in data collection and supervision. RD helped in the concept, data collection, processing, and analysis. JP as a main guide developed the concept, did the literature review, helped in data collection, processing, analysis, and clinical review. All authors read and approved the final manuscript.

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Availability of data and materials

Available with the Himachal Pradesh University, Department and with CTRI as thesis submitted for postgraduation degree for MD anaesthesia.

Declarations

Ethics approval and consent to participate

Ethical no: HFW(MC-11) B (12) ETHICS/2020/13936/dated 13/8/2020 Shimla (ethical committee of the institution IGMC which is a registered. CTRI No: 2020/10/028448. All patients included had consented to be part of the research.

Consent for publication

All authors have consented for publication. Patient's consent was taken in English or vernacular language as applicable on the informed consent form. The written informed consent to publish this information was obtained from study participants. All patients included had consented to be part of the research and thereafter publication of data.

Competing interests

The authors declare that they have no competing interests.

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