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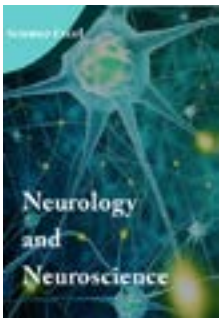

Pituitary adenomas and COVID-19 related disease in pandemic time: Clinical, pathological, immunohistochemical, and ultrastructure analysis

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Keywords

Pituitary adenoma, Pituitary apoplexy, COVID-19, endothelial cells, vascular injury.

Abbreviations

PA: Pituitary Apoplexy; ST: Sella Turcica; PAD: Pituitary Adenoma; PG: Pituitary Gland; FPAD: Functional Pituitary Adenoma; NFPAD: No Functional Pituitary Adenomas; SAH: Subarachnoid Hemorrhage; DM: Diabetes Mellitus; PDGF: Platelet-Derived Grow Factor; EVGF: Endothelial Vascular Growth Factor; Col IV: Collagen IV; GFAP: Glial Fibrillary Acidic Protein; CNS: Central Nervous System; NRP: Neuropilin; VEGF: Vascular Endothelial Growth Factor; MVD: Microvascular Density; Flk-1: Fetal Liver Kinase 1; TNF-α: Tumor Necrosis Factor-α; PTTG: Pituitary Tumor-Transforming Gene; MMP-2/9: Matrix Metalloproteinase-2/9; Ki-67: Proliferating Marker; DDR1: tyrosine kinase receptor discoidin domain receptor l; ACE2: Angiotensin-Converting Enzyme Type 2 Ang-II; CoV: Coronavirus; HPA: Hypothalamic-Pituitary-Adrenal; RAS: Renin-Angiotensin System; SARS: Severe Acute Respiratory Syndrome

Abstract

Introduction:

Methods: 47 patients were emergency operated on due to pituitary apoplexy during the pandemic time. The patients were divided into two groups according to PCR COVID-19 positive vs negative test. Histopathology all cases showed varying degrees of necrosis, microthrombi formation secondary to inflammation, and endothelial cells injuries in association with TNFa, TNFk, FVIII, DPGF, HIF1a, Il6, Il10, Il17, DPGF, CD3, CD4, CD8, CD20, CD68, CD163, ACE2, and antiCOV immunoexpression.

Results: 24 women and 23 men, age ranges from 21 to 76 years (mean 42.25±13.38), 14 (36%) presented positive COVID19 tests, and 30 (64%) were covid negative. 15 were already recurrences, 4 recurrences in the same year, and 4 died during the pandemic time. 9 (30%) showed histological data of stroke, of which necrosis around <25% were 8 (47%), 25-50% were 2 (12%) and >50% were 8 (45%). Weak vascular changes in 4 (24%), moderate in 7 (65%) and severe in 1, moderate, and intense in 1 (6%) were observed.

Conclusions: Apoplexy in a previously diagnosed macro-PAD in the setting of a recent COVID-19 infection. The patients who presented with histological features of pituitary tumor infarction alone had less severe clinical features on presentation, a longer course before presentation, and a better outcome than those presenting with hemorrhagic infarction or frank hemorrhage. Hyperactivated and dysregulated immune cells pose a substantial danger for exacerbated tissue damage. COVID-19 may increase the risk of pituitary apoplexy, and we should be vigilant for signs of this. A more insidious pathological link between COVID-19 and apoplexy may exist in addition to severe inflammatory response.

Introduction

The classical term "pituitary apoplexy" (PA) [1] denotes a clinical syndrome characterized by abrupt onset of headache accompanied by neurologic or endocrinologic deterioration due to a sudden expansion of a mass within the sella turcica as a consequence of hemorrhage, infarction, or necrosis within a pituitary tumor (PT) and adjacent pituitary gland (PG) [2]. Typical symptoms of apoplexy include sudden onset of headache, visual impairment, diplopia, nausea, vomiting, fatigue, and hormonal deficiencies. The incidence of PA varies from 0.6 to 22% with different diagnostic criteria [3]. It has been associated with a variety of co-morbidities including hypertension, diabetes, and anticoagulation therapy [2,3], and most recently with the coronavirus disease 2019 (COVID-19) [4]. Since the first identification of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019, in China, the virus has spread rapidly throughout the globe, causing severe morbidity and mortality of SARS-CoV-2 infection (COVID-19) [5]. The infection in the central nervous system (CNS), causing a multitude of neurological manifestations such as acute cerebrovascular diseases, encephalopathy, acute polyneuropathy, hypogeusia, encephalitis, and hyposmia, as well as certain non-specific symptoms (headaches, myalgia, fatigue, unsteadiness, etc) [5-7]. It is strongly associated with thrombosis: thrombosis in large vessels, deep vein thrombosis, embolism, arterial events, and possible microvascular thrombosis may occur [5-7]. Several reports in humans and animal models showed a significant angiotensin-converting enzyme type-2 (ACE2) mRNA expression in hypothalamus and pituitary cells [7]. Fleseriu

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et al. [4] recommend cataloging COVID-19 positive patients into essential pituitary surgery as either emergent, urgent, or elective regarding operative care recommendations [4]. Surgical removal of the pituitary lesion is straightforward. However, due to the patient's COVID-19 status, complete respiratory and contact-related precautions have been carried out [5]. Moreover, higher mortality and poorer outcomes have been widely described in COVID-19 patients with obesity, diabetes and vertebral fractures, which are all highly prevalent in subjects with pituitary dysfunctions. Endocrine manifestations of COVID-19 with their possible implications for pituitary diseases, the possible direct and indirect involvement of the pituitary gland in COVID-19, the impact of COVID-19 on the management of established pituitary diseases which can be already at increased risk for worse outcomes. Moreover, the care for pituitary diseases need to continue despite the restrictions due to the emergency.

This work aimed to study the pituitary adenomas (PAD) and PA related or not with patients with COVID-19. Clinical, pathological, immunohistochemical, immunofluorescence, and electron microscopy analysis were also performed.

Methods

Patients attended the Neuropathology Department affiliated with the National Institute of Neurology and Neurosurgery of Mexico City. This work included all patients diagnosed and operated on with Pituitary Tumors from January 1st,2020 to December 31st, 2020. All patients had operations either trans-sphenoidal microsurgery or craniotomy. 47 cases were recruited. The research had achieved authorization from the Ethics Committee of the First Affiliated institution (No.22-21).

Data collection

Data on clinical features including sex, age, main complaint, clinical manifestations, results of radiological imaging examination, endocrine function tests, pathological results, and medical history, PCR COVID-19 test were obtained from the medical record database in all patients.

Cases and controls

The factors that influence the incidence of pituitary apoplexy were studied in a matched case-control study. Patients with pituitary apoplexy with COVID-19 (case group) were contrasted with pituitary apoplexy COVID-19 negative (control group). Patients of the control group were nominated by a computer-generated random selection scheme. Control patients were harmonized with case-patients by age, and functional vs non-functional adenoma.

Diagnostic criteria

Patients who arrived at the emergency service at our Institution were included. Due to pandemic contingency, only emergency cases were operated during this year (2020). Patients with clinical and radiological criteria of pituitary apoplexy were operated on. Noticeable symptoms of PA (sudden headache, visual impairment, nausea, or vomiting), as well as, the radiologic signs of hemorrhage, intraoperative or pathological features of hemorrhage and necrosis, were analyzed.

Imaging examination

Computed tomography (CT) and/or magnetic resonance imaging (MRI) scans were performed on all patients. Three-dimensional imaging provided by MRI shows the location and the degree of hemorrhage. Hardy-Vezina and Knosp classifications [8]. Inflammation, and necrosis were evaluated.

Pathology examination

Percentage of necrosis and hemorrhage (negative, 1-10 % (1), 10-30 % (2), 30-50 % (3), and >50 % (4)), presence or absence of polymorphonuclear cells, macrophages, endothelial necrosis, or hyperplasia of endothelial cells, vasculitis, fintra and extravascular deposition of fibrin. Droplets of lipid and edematous macrophages and hemosiderophages and hemosiderin depositions were also analyzed in relation with functional vs non-functional adenomas vs COVID-19 positive or not.

Immunohistochemical staining techniques

Standard immunohistochemical staining was used to determine vascular damage, inflammation, and cytokines production according to functional vs nonfunctional tumor and tumor size, to define vascular changes in pituitary apoplexy related or not with patients with positive COVID-19. The average numbers of infiltrating cells were classified semi-quantitatively as follows: less than 20 (weak), 20-40 (moderate), and more than 40 (severe).Tissue samples were fixed in 4% formalin, embedded in paraffin, and sliced into 4 mm-thick sections.

The slides were stained with an auto-immune stainer using the following monoclonal antibodies: CD31 (M0823), and CD34(M7165, Dako Cytomation; Glostrup, Denmark, 1;100), Factor VIII(MU016-UC,BIOGENEX, dilution 1;100),Tumoral necrosis factor alpha antibody protein[TNFα/1172] (GTX35131, dilution 1.100), TNFβ,(antirabbit, Anti-TNF beta antibody (GTX26681) | GeneTex, dilution 1:100), interleukin 6 [(IL6) rabbit polyclonal antibody (GTX110527) | GeneTex, dilution 1:100), interleukine 10 (IL-10 Anticuerpo (A-2): sc-365858, Il7(IL-17 Anticuerpo (E-19): sc-6077 - Santa Cruz Biotechnology, dilution 1.100), platelet-derived grow factor [Factor -AB (PDGF-AB), B-chain (36t) Aptamer(Cat#: CTApt-047, dilution 1;100), CD68(Anti-CD68 Antibody (E-11) | SCBT - Santa Cruz Biotechnology,dilution 1:100), CD163((sc-33560) CD163 Antibody (M-96) - Santa Cruz Biotechnology, dilution 1,100), CD3(MU322-UC, BIOGENEX,dilution 1:100), CD4 (MU421-UC, BIOGENEX, dilution 1; 100), CD8 (MU422-UC,BIOGENEX, dilution 1;100), CD20 (CM004C BIOCARE, dilution 1:100),hypoxic inductor factor 1[(Hif1) HIF-1α antibody (28b) SCBT - Santa Cruz Biotechnology], rabbit primary antibody for ACE2 (1:200, ab108252). (Coronavirus Anticuerpo (FIPV3-70): sc-65653, dilution 1:100). IHC was conducted using the BioSB Kit procedures (Biotin-Streptavidin AP or HRP, 2-Step Immunohistochemistry Detection Technology. Santa Barbara CA, USA). Finally, the slides were developed with DAB and counterstained with hematoxylin.

Ultrastructural examination

Electron microscopy was also performed in embedded blocks of paraffin to evaluate the endothelial injury and the presence of SARS COV-2 virions. Transmission electron microscope (Jeol microscope) and digital images and measurements were acquired using AMT image capture software (version 602.446).

Statistics analysis

All data were processed and analyzed using SPSS software (V. 25.0; SPSS Inc., Chicago, IL, USA). Continuous variables were analyzed by independent t-test and categorical variables by crosstab analysis. Statistical significance was defined as $p<0.05$.

Results

All patient presented with clinical signs which were borderline for meeting criteria for hospitalisation, indicating a more moderate course of disease. This suggests that vascular dysfunction was not the likely major mechanism leading to apoplexy, although understanding that the vascular fragility of the pituitary gland increases this risk. We performed a retrospective study involving 47 patients; 24 (51%) were female and 23(49%) were males. The mean range of the age was from 21 to 76 years (42.67±13.216). The median age for both genders was 39 years. The median age of patients presenting with PA was 46.7 years (IQR 31.5–57.0 years). The follow-up range for women was 12-120 months (mean of 34.08±5.307) and for men, it was 3-120 months (mean of 39.76±7.648). 17 (36%) were FPAD and 30 (67%) were NFPAD. 17 (36%) presented clinical histological data of COVID-19 and 30 (64%) cases did not. 15 cases recurred and 32 did not, of which 8 recurred in pre-pandemic times. 3 patients died during this time. Of 17 cases with positive COVID-19 tests, only 9 (53%) presented stroke histologically (p=.009). The clinical and epidemiological characteristics of the patients are seen in Table (1).

Radiologically: Of 17 patients covid test positive. One was HV Grade I, 9 (53%) were grade III, and 6 (35%) were grade IV (Figure 1). Obsevig within the pituitary gland, extending upwards into the suprasellar cistern compressing the optic chiasm. The MRI appearances were consistent with haemorrhage into a pre-existing pituitary macroadenoma, confirming pituitary apoplexy.

Table 1. *Clinical data of the patients studied.*

	COVID-19			PITUITARY APOPLEXY			FUNCTIONAL ADENOMA		
CLINICAL VARIABLE	YES (N=17)	NO (N=30)	p Value	YES (N=14)	NO (N=33)	p Value	YES (N=17)	NO (N=30)	p Value
Gender Femenine Male	9 (53) 8 (47)	15 (50) 15 (50)	0.846	6 (43) 8 (57)	18 (55) 15 (45)	0.464	11 (65) 6 (35)	13 (43) 17 (57)	0.159
Functional Adenoma	9 (53)	8 (27)	0.072	6 (43)	11 (33)	0.534	----	-----	----
Pituitary Apoplexy	9 (53)	5 (17)	0.009	----	----	----	6 (35)	8 (27)	0.534
Headache	14 (82)	21 (70)	0.351	13 (93)	22 (67)	0.06	11 (65)	24 (80)	0.248
Amaurosis	13 (76)	22 (73)	0.813	11 (79)	24 (73)	0.674	10 (59)	25 (83)	0.064
Hardy Vezina 0 I II III IV	1 (6) 1 (6) 0 (0) 9 (53) 6 (35)	1 (3) 0 (0) 1 (3) 17 (57) 11 (37)	0.639	0 (0) 0 (0) 0 (0) 8 (57) 6 (43)	2 (6) 1 (3) 1 (3) 18 (55) 11 (33)	0.744	2 (12) 1 (6) 0 (0) 9 (53) 5 (30)	0 (0) 0 (0) 1 (3) 17 (57) 12 (40)	0.183
Suprasellar Extension 0 A B C D E	1 (6) 1 (6) 2 (12) 3 (18) 6 (35) 4 (24)	1 (3) 0 (0) 3 (10) 10 (33) 11 (37) 5 (17)	0.669	0 (0) 0 (0) 1 (7) 3 (21) 5 (36) 5 (36)	2 (6) 1 (3) 4 (12) 10 (30) 12 (36) 4 (12)	0.461	2 (12) 1 (6) 1 (6) 4 (24) 6 (35) 3 (18)	0 (0) 0 (0) 4 (13) 9 (30) 11 (37) 6 (20)	0.3
Recurrence	4 (24)	4 (13)	0.371	4 (29)	11 (33)	0.745	3 (18)	5 (17)	0.932
Death	3 (18)	0 (0)	0.017	3 (21)	0 (0)	0.006	2 (12)	1 (3)	0.256
Hypogonadism	8 (47)	10 (33)	0.352	7 (50)	11 (33)	0.282	7 (41)	11 (37)	0.76
Hypothyroidism	14 (82)	26 (87)	0.69	13 (93)	27 (82)	0.331	14 (82)	26 (87)	0.69
Hypocortisolism	8 (47)	19 (63)	0.278	8 (57)	19 (58)	0.978	7 (41)	20 (7)	0.089
Hypopituitarism	10 (59)	18 (60)	0.937	10 (71)	18 (55)	0.281	9 (53)	19 (63)	0.485
Insipidus Diabetes	3 (18)	16 (53)	0.017	5 (36)	14 (42)	0.668	6 (35)	13 (43)	0.589
Post-surgical Fistula	2 (12)	5 (17)	0.65	3 (21)	4 (12)	0.412	4 (24)	3 (10)	0.211
Radiotherapy	3 (18)	2 (7)	0.241	1 (7)	4 (12)	0.613	1 (6)	4 (13)	0.426
Tabaquism	4 (24)	6 (20)	0.776	5 (36)	5 (15)	0.115	3 (18)	7 (23)	0.647
COVID-19	----	----	-----	9 (64)	8 (24)	0.009	9 (53)	8 (27)	0.072

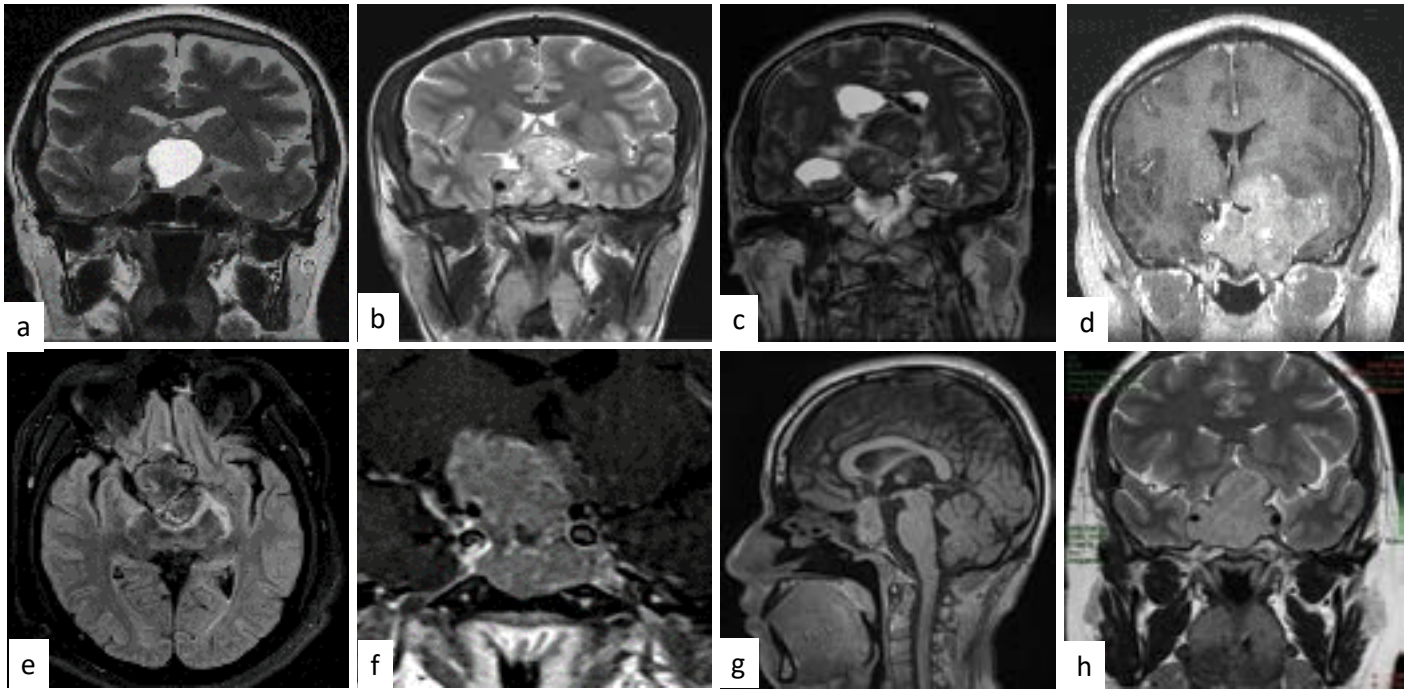

Figure 1. *Pituitary Macroadenoma HV III-B/Knosp 1 and Pituitary Apoplexy, MRI T1+C shows a hyperintensity lesion in the sellar region, of heterogenous component, that widens and erodes the sella turcica in its anterior portion, with the presence of hemorrhage within and volume of approx. 1.3 cm (a), which comprises the optic chiasma and contacts with the left cavernous sinus (b and c).*

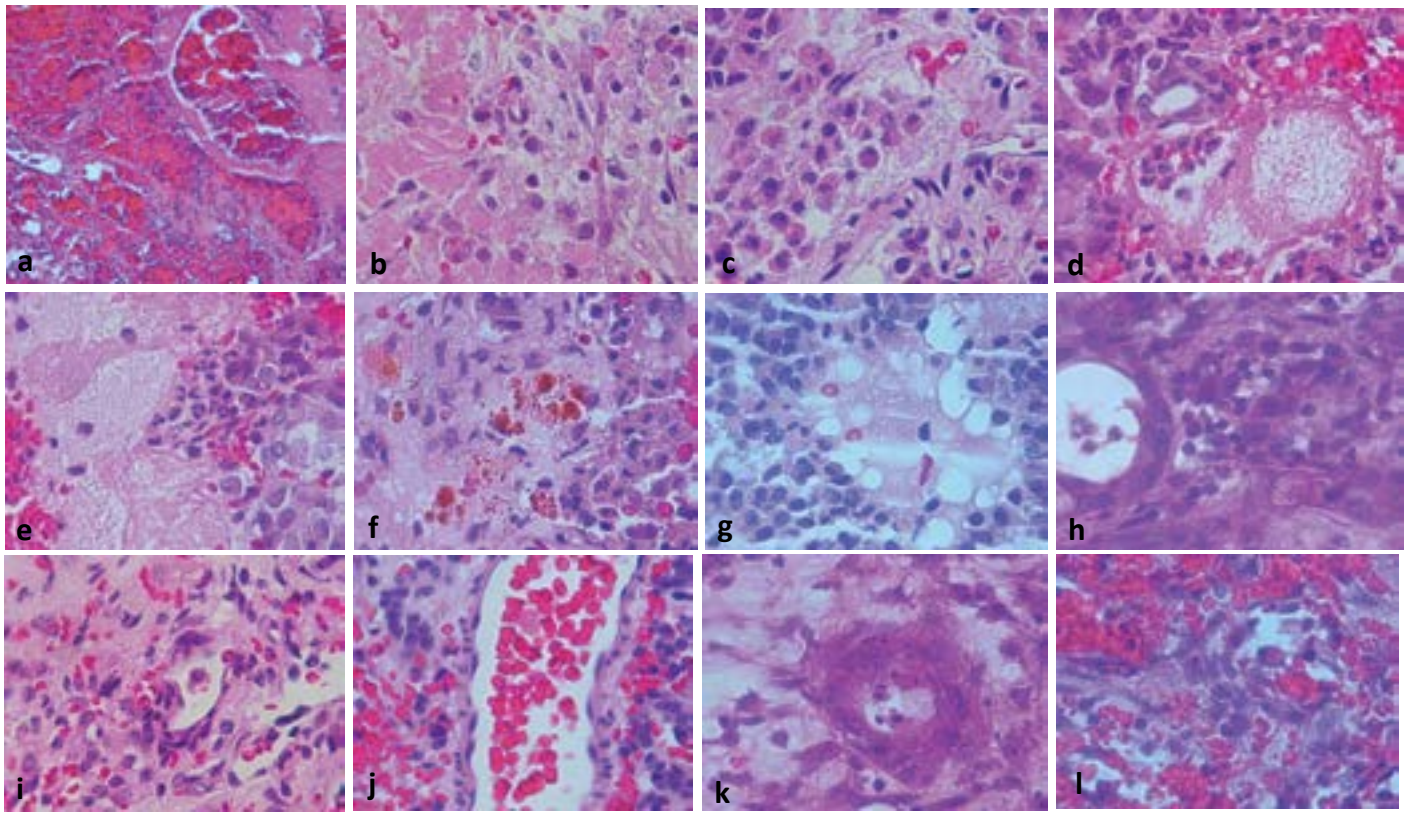

Figure 1. *Histopathological features observed vascular injuries; (a) necrosis of and hemorrhage, (b) individual necrosis, and (c) oncocytic appearance. (d) Necrosis and edema, (e) necrosis and polymorphonuclear cells in (f) showed hemosiderophages and in (g) lipidic or droplets of lipids with a vacuolated or sparkling appearance. (h) Endothelial cells damages showed hyperplasia of endothelial cells and perivasculitis, (i) and (j) showed hyperchromatic endothelial cells and loss of them. In (k) disorder of the vessel layers due to inflammatory cells. In (l) observes necrosis and debris of endothelial cells (H&EX400, original magnifications).*

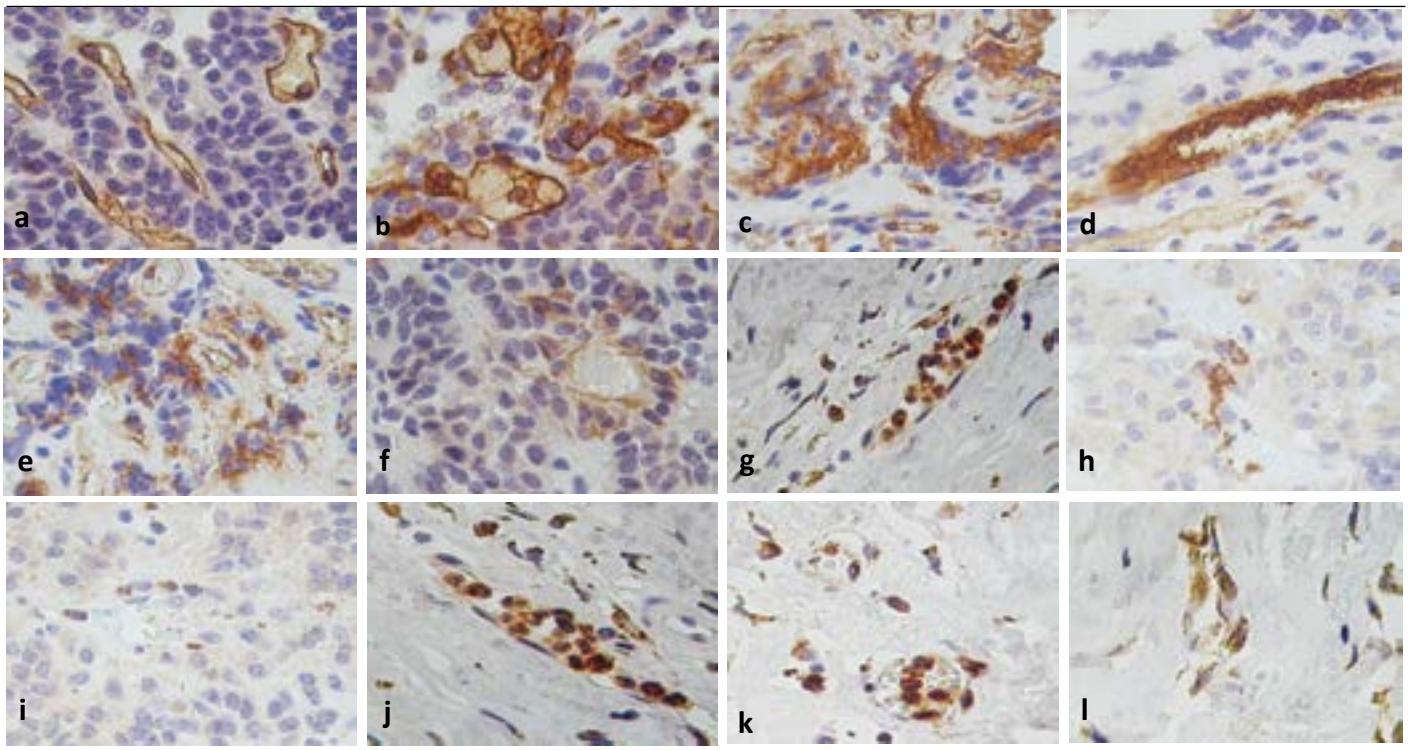

Figure 3. *Immunohistochemistry. (a) we observe the endothelial cells are positive for CD34, (b) CD31 we observe endothelial cell hyperplasia, these become large with abundant cytoplasm. (c) FVIII in vessels shows a densely positive granular material. (d) positive endothelial cells for PDGF. (e)TNFa positive immunoreaction, (f) Interleukin 6 positive reaction in ECs, and (g) iL17 was focal positive immunoreaction. (h) CD68 positive, (i) CD163 was mild in perivascular form. (i) CD8 positive in the wall and perivascular lymphocytes exclusively. (j) ACE2 and in (k) COV granular positive immunoreaction (IHQ stain x400 original magnifications).*

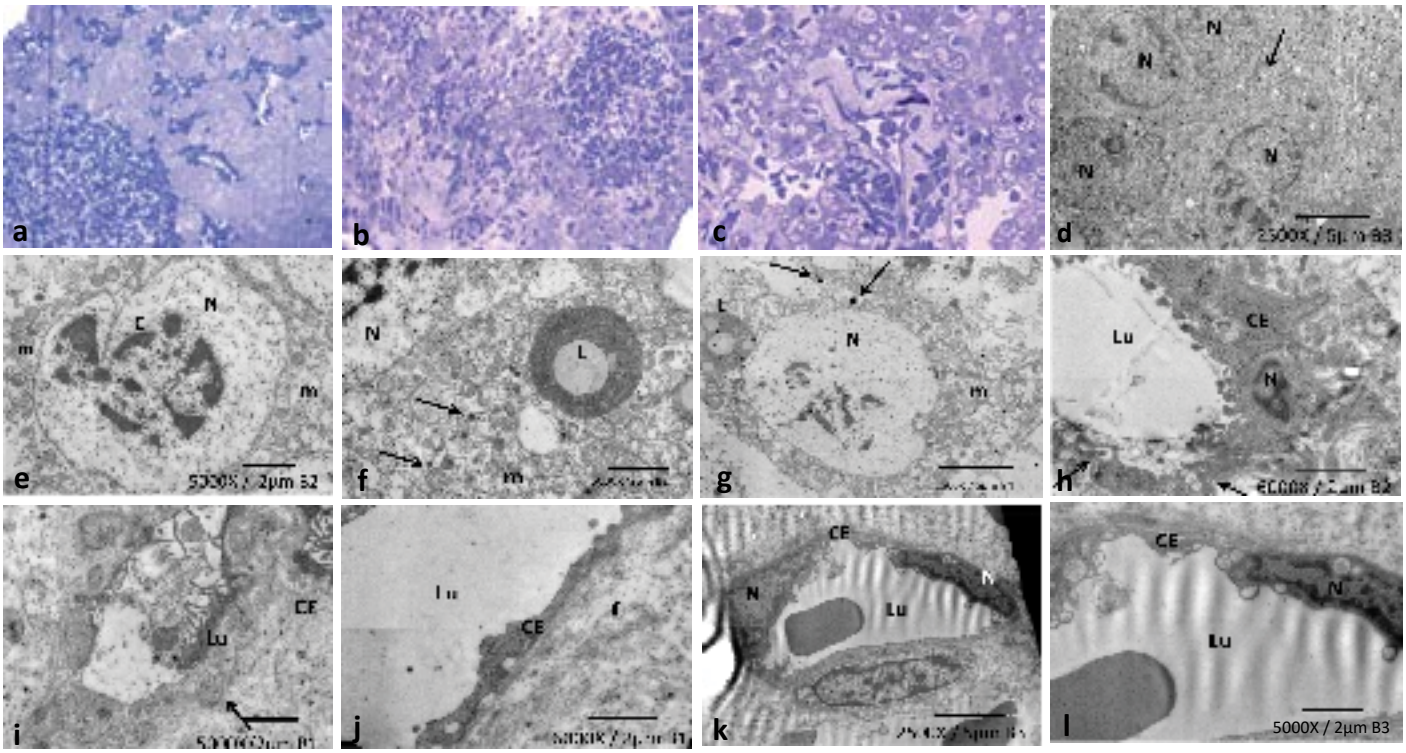

Figure 4. *Ultrastructure features. (a) Semifine court showed necrosis, (b) perivascular inflammation, and in (c) endothelial cell damage (blue toluidine stain x100). The ultrastructure image showed pituitary adenoma cells. (d) few preserved cells showing nuclei and secretory granules (arrow). (2500X / 5µm B3). And in (e) Necrotic cells show nuclear alterations with edema and chromatin arranged in electron-dense clusters. There are oncocytic changes denoted by a large number of mitochondria (m)(5000X / 2µm B2). (f) and (g) necrosis cell changes were observed. Secretory granules (arrows), nuclei with edema, oncocytic changes with a large number of mitochondria, presence of abnormal lysosomes (2500X / 5µm B4) are observed. In (h) the vessel wall that has lost its structure, a thin elastic lamina (arrow) is observed (6000X / 2µm B2) and in (i) Destruction of the vessel with loss of the endothelial cell layer is observed (5000X /2µm B1). (j) A thin layer of endothelial cells and remains of the muscle layer fibers (6000X / 2µm B1), (k) (2500X / 5µm B3), and (l) (5000X / 2µm B3), showed endothelial cells Damage, they have lost their structures.*
N- nucleus, C.- chromatin, lysosomes (L). - endothelial cells (CE), lumen (lu).

Table 2. *Histopathology figures in association with COViD19 positive test, pituitary adenomas and functional adenomas.*

	COVID-19			PITUITARY APOPLEXY			FUNCTIONAL ADENOMA		
HISTOLOGICAL VAREABLES	**YES (N=17)**	**NO (N=30)**	**p Value**	**YES (N=14)**	**NO (N=33)**	**p Value**	**YES (N=17)**	**NO (N=30)**	**p Value**
Necrosis									
Negative	5 (30)	6 (20)		2 (14)	9 (27)		4 (24)	7 (23)	
10%	1 (6)	14 (47)		4 (29)	11 (33)		5 (30)	10 (33)	
25%	8 (47)	5 (17)	0.034	5 (36)	8 (24)	0.54	5 (30)	8 (27)	0.887
50%	2 (12)	4 (13)		2 (14)	4 (12)		3 (18)	3 (10)	
>50%	0 (0)	1 (3)		0 (0)	1 (3)		0 (0)	1 (3)	
Total	1 (6)	0 (0)		1 (7)	0 (0)		0 (0)	1 (3)	
Hemorrhage									
Negative	1 (6)	4 (13)		2 (14)	3 (9)		1 (6)	4 (13)	
Weak	10 (59)	20 (67)	0.416	8 (57)	22 (67)	0.791	12 (71)	18 (60)	
Moderate	6 (35)	5 (17)		4 (29)	7 (21)		4 (24)	7 (23)	0.724
Intense	0 (0)	1 (3)		0 (0)	1 (3)		0 (0)	1 (3)	
Inflammation									
Negative	1 (6)	13 (43)		2 (14)	12 (36)		3 (18)	11 (37)	
Weak	4 (24)	17 (57)	0	5 (36)	16 (48)	0.056	7 (41)	14 (47)	0.136
Moderate	11 (65)	0 (0)		6 (43)	5 (15)		7 (41)	4 (13)	
Strong	1 (6)	0 (0)		1 (7)	0 (0)		0 (0)	1 (3)	
Vascular Changes									
Negative	4 (24)	17 (57)		4 (29)	17 (52)	0.442	8 (47)	13 (43)	0.538
weak	7 (41)	8 (27)	0.168	6 (43)	9 (27)		4 (24)	11 (37)	
Moderate	4 (24)	3 (10)		2 (14)	5 (15)		4 (24)	3 (10)	
Strong	2 (12)	2 (7)		2 (14)	2 (6)		1 (6)	3 (10)	
Edema									
Negative	5 (30)	15 (50)		4 (29)	16 (48)	0.33	8 (47)	12 (40)	0.895
Weak	8 (47)	13 (43)	0.171	7 (50)	14 (42)		7 (41)	14 (47)	
Strong	4 (24)	2 (7)		3 (21)	3 (9)		2 (12)	4 (13)	
Invasion									
Negative	10 (59)	17 (57)	0.988	6 (43)	21 (64)	0.404	11 (65)	16 (53)	0.744
Weak	6 (35)	11 (37)		7 (50)	10 (30)		5 (30)	12 (40)	
Moderate	1 (6)	2 (7)		1 (7)	2 (6)		1 (6)	2 (7)	
Atypia									
Negative	5 (30)	13 (43)		3 (21)	15 (45)		6 (35)	12 (40)	
Weak	8 (47)	10 (33)	0.734	6 (43)	12 (36)	0.407	5 (30)	13 (43)	0.147
Moderate	3 (18)	6 (20)		4 (29)	5 (15)		6 (35)	3 (10)	
Strong	1 (6)	1 (3)		1 (7)	1 (3)		0 (0)	2 (7)	
Mitosis figure	5 (29)	6 (20)	0.464	5 (36)	6 (18)	0.194	7 (41)	4 (13)	0.03
Lipid Material									
Negative	5 (30)	12 (40)		4 (29)	13 (39)		7 (41)	10 (33)	
Weak	6 (35)	14 (47)	0.107	6 (43)	14 (42)	0.517	7 (41)	13 (43)	0.566
Moderate	3 (18)	4 (13)		2 (14)	5 (15)		3 (18)	4 (13)	
Strong	3 (18)	0 (0)		2 (14)	1 (3)		0 (0)	3 (10)	

	COVID-19			PITUITARY APOPLEXY			FUNCTIONAL ADENOMA		
HISTOLOGICAL VAREABLES	**YES (N=17)**	**NO (N=30)**	**p Value**	**YES (N=14)**	**NO (N=33)**	**p Value**	**YES (N=17)**	**NO (N=30)**	**p Value**
Polymorphonuclears									
Negative	4 (24)	12 (40)		3 (21)	13 (39)		3 (18)	13 (43)	
weak	6 (35)	16 (53)	0.002	8 (57)	14 (42)	0.422	10 (59)	12 (40)	0.145
Moderate	7 (41)	0 (0)		3 (21)	4 (12)		4 (24)	3 (10)	
Strong	0 (0)	2 (7)		0 (0)	2 (6)		0 (0)	2 (7)	
Haemosiderin									
Negative	5 (30)	9 (30)		4 (29)	10 (30)		6 (35)	8 (27)	
weak	9 (53)	16 (53)	0.979	9 (64)	16 (48)	0.333	8 (47)	17 (57)	0.886
Moderate	2 (12)	4 (13)		0 (0)	6 (18)		5 (30)	4 (13)	
Strong	1 (6)	1 (3)		1 (7)	1 (3)		1 (6)	1 (3)	
MGravas									
Negative	6 (35)	13 (43)		4 (29)	15 (45)		8 (47)	11 (37)	
weak	7 (41)	13 (43)	0.291	8 (57)	12 (36)	0.462	6 (35)	14 (47)	0.848
Moderate	4 (24)	2 (7)		2 (14)	4 (12)		2 (12)	4 (13)	
Strong	0 (0)	2 (7)		0 (0)	2 (6)		1 (6)	1 (3)	
Vasculitis									
Negative	2 (12)	15 (50)		2 (14)	15 (45)		5 (30)	12 (40)	
Weak	12 (71)	12 (40)	0.043	11 (79)	13 (39)	0.1	11 (65)	13 (43)	0.486
Moderate	2 (12)	3 (10)		1 (7)	4 (12)		1 (6)	4 (13)	
strong	1 (6)	0 (0)		0 (0)	1 (3)		0 (0)	1 (3)	

Histological features associated with COVID-19 positive vs COVID-19 negative (p=.009), results are seen in Table 2 (Figure 2). We observed vascular injuries (a), necrosis and hemorrhage (b), individual necrosis (c), and oncocytic appearance (d). Necrosis and edema (e), necrosis and polymorphonuclear cells (f), hemosiderophages (g), lipidic or droplets of lipids with a vacuolated sparkling appearance (h). Endothelial cells damages showed hyperplasia of endothelial cells and perivasculitis (i), and hyperchromatic endothelial cells and loss of them (j). as well as, the derangement of the vessel layers by inflammatory cells is shown (k). (l) also, necrosis (apoptosis) and debris of endothelial cells were observed (j).

By immunohistochemistry (Figure 3), we observe the endothelial cells positive for CD34 (a), CD31 positive reaction observed hyperplasia of endothelial cells, these become large with abundant cytoplasm. FVIII positive reaction in vessels showed a densely positive granular material (c). PDGF was also positive in endothelial cells (d). TNFα positive immunoreaction was positive in EC and in some astrocyte (e), Interleukin 6 (f), and iL17 (g) were positive reaction in ECs positive immunoreaction. CD68 was positive in macrophages between necrosis zones as well as in tumor cells (h). CD163 was weak in perivascular form (i). CD3 and CD4 were negative and CD8 was positive exclusively in vascular walls and perivascular lymphocytes. ACE2 was positive in vascular wall in ECs as well as, in occasional inflammatory cells (j) and COV positive immunoreaction was granular in occasional inflammatory cells and macrophages (k). CD20 was negative.

By electron microscopy (Figure 4), aggregates of uniform, round enveloped particles ranging in size from around 70 nm to 100 nm with peripheral spike-like projections consistent with the morphology described for SARS-CoV-2 were observed. The ultrafine court showed necrosis (a) perivascular inflammatory cells (b) and endothelial cell damage (c). Epithelial cell damage is seen in (d, e, f). Endothelial cell damage was also observed (g-l).Hipoxic isquemic changes was observed.

Clinicopathological correlation

In our work, there was no correlation between clinical data with patients with COVID-19 who presented with functional vs non-functional stroke. Histologically, there was a statistically significant difference between positive COVID-19 with inflammation (p=.000) and with the presence of polymorphonuclear leukocytes (p=.002). By immunohistochemistry, there were statistically significant differences in the expression of CD3, CD20, CD68, CD163, TNFα, TNFκ, iL6, iL10, GFAP, and s100 (p=.004 respectively).

Discussion

Pituitary apoplexy (PA) is a medical and surgical emergency due to its association with both hormonal dysregulations, in addition to cerebral ischemia, subarachnoid hemorrhage, brain strokes, and death. Rapid recognition and treatment are consequently vital [1]. In the vast existing literature on COVID-19 infection, few cases of pituitary necrosis and apoplexy have been published [9,10], which propose a link between pituitary apoplexy and concomitant or preceding COVID-19 infection. It has been associated with a variety of co-morbidities including hypertension, pregnancy, diabetes, obesity, anti-coagulation therapy, major surgery, and head trauma [3]. Apoplexy has been associated with pregnancy in several series, although the relationship between these two occurrences is unclear [4]. Endocrinal disturbance has been also reported in association with COVID-19 infection and PA [11,12], while this remains speculative at this point, is known to trigger

or unleash endocrinopathies both via autoimmune mechanisms and through organ damage [12]. Both ACE2 and TMPRSS2 are widely expressed in many endocrine glands. This, along with several case reports of thyroid and pituitary disruption in patients with COVID-19, has resulted in significant interest in its impact on the endocrine system [11]. The relationship between devastating infection provoking both stress and inflammatory response leading to vascular dysfunction and predisposing to cerebrovascular events can reasonably be speculated on [10-13].

One of the maximums clinically pertinent consequences of the COVID-19 pandemic for pituitary patients is inadequate and restricted access to surgery since being more vulnerable to COVID-19 [14]. There is a general risk linked to their hospitalization as well as specific risk resultant from endoscopic endonasal surgery which due to the access via the nasal cavity, paranasal sinuses, or mastoid air cells was considered a high-risk procedure [4,14]. In fact, in neurosurgical units, urgent interventions were prioritized, over those with benign lesions, and usually were cases of PA.

The MR imaging findings were able to predict the histopathology accurately in most of our cases. The group of patients with PAD had less severe clinical features and a better outcome than those with hemorrhagic infarction/hemorrhage [2].

The pathogenesis of pituitary apoplexy is not completely understood. It is unclear whether the COVID-19 infection is a contributing factor in the apoplectic event [8]. The role of ACE2 on normal pituitary or PitNET (pituitary adenomas) is yet to be well elucidated. The expression and distribution of ACE2 indicate the risk and severity of SARS-CoV-2 as a potential impact on corticotrophs [12]. It has been observed an increase in serum PRL and ACTH in patients compared to healthy individuals [12], this might be accredited to dysfunction, such as the possible injury of corticotrophs instead of stress responses or feedback regulation of the adrenal axis. Therefore, the current data provide evidence that the hypothalamic-pituitary-adrenal (HPA) axis is started in non-critical COVID-19 patients but suppressed in complicated patients by modulating a multitude of homeostatic processes, including immune defense mechanisms, inflammation, and cellular metabolism [11,12].

Systemic involvement in COVID-19 is due to the ubiquitous expression of angiotensin-converting enzyme 2 (ACE2) receptor, responsible for the entry in the cells of SARS-CoV-2. ACE2 mRNA expression was reported also in hypothalamus and pituitary gland cells, as occurs in an infectious stress state, it may increase pituitary blood demand precipitating acute apoplexy [10,11]. It is a risk factor for pituitary apoplexy since SARS-CoV-2 can induce thrombocytopenia, coagulopathy, and platelet dysfunction, having neural tissue tropism due to ACE2 expression in cerebral vascular endothelium [13]. Moreover, the virus has been theorized to enter the brain by the nasopharyngeal epithelium via the olfactory nerve or might pass through the blood-brain barrier or straight reach the median eminence, a circumventricular organ where the BBB is missing [13]. Infection-induced thrombocytopenia, platelet dysfunction, coagulopathy, and immune-mediated hypophysitis have been documented as precipitants for PA [13]. Owing to COVID-19 patients are at increased risk for endothelial injury, causing microthrombi, which has become central to the discussion of patient management [1-2,15].

The vascular endothelium, building the inner layer of capillaries and blood vessels of all sorts, represents a highly active metabolic and endocrine organ producing a multitude of different molecules, including vasoactive peptide hormones, growth factors, coagulation factors, and adhesion molecules [7-8]. Moreover, it expresses many of the respective endocrine, paracrine, and cytokine/growth factor receptors [15,16]. It thereby regulates the delicate balance between vasoconstriction and vasodilation; coagulation and fibrinolysis, endothelial tumefaction in pituitary capillaries and fibrinous thrombi in small arterioles, proliferation, and apoptosis, as well as between transient adhesion and diapedesis of blood-borne leukocytes [12-16].

Tumor hypoxia, following rapid tumor growth, may promote hemorrhagic transformation in pituitary adenomas via the HIF-1α signaling pathway [17]. Angiogenesis has been quantified by immunohistochemical expression of Flk-1, NRP, CD31, CD34, FVII, and VEGF expression, while MVD has been analyzed in PA. However only DPGF is positively correlated with both radiological and pituitary hemorrhage, and a positive correlation was also identified between angiogenesis and MVD [10]. Endothelial cell injuries as well as, endarteritis or vasculitis, have not been fully studied in PA. EVGF, TNF-α, PTTG, MMP-2/9), Ki-67, along HIF1a are the major contributing factors involved in pituitary apoplexy [11,17]. The overexpressed DDR1 protein further increased the expression of MMP-2/9 which caused hemorrhagic/infarction of the pituitary adenoma [17]. Angiogenesis is a fundamental process for the development and growth of a tumor and is less expressive in adenomas than in the normal pituitary tissue. However, in PA the MDV is higher [18]. However, age, gender, tumor size, hormonal functioning, cyst formation, and cavernous sinus invasion had no relationship with VEGF expression [18].

Angiopoietin-2 is a molecule responsible for angiogenesis that increases vascular permeability by antagonizing the action of angiopoietin-1 and cancer progression, which correlates with tumor load in certain cancers, is upregulated by the angiotensin 2-AT1 Receptor axis [17].

COVID-19 CNS-related disorders include hemorrhage in the context of thrombocytopenia, disseminated intravascular coagulation, endothelial cell damage [9,10], etc. Thrombi and thromboembolic events have been suggested to be caused by an effect of the virus on the vascular walls, patchy thrombi in microvessels, and segregation of thrombocytes and neutrophils granulocytes in the vessel. Focal necrosis, neutrophilic plugs, neuronophagia, and microglial nodules [16] cytopathic effect (CPE)[18-20]. However, the observation of virally infected endothelial cells has been called into question [21,22].

The tumor microenvironment (TME) consists of extracellular matrix (ECM), macrophages, fibroblasts, endothelial cells, inflammatory cells [23], etc. TME is associated with various steps in cancer progression which include initiation, progression metastasis, modulation of the ECM, and stimulating cancer cell growth by releasing anti-inflammatory cytokines and growth factors [23]. The growth of the adenoma and secondary hemorrhage could be an event directly related to the TME and inflammatory factors: which neurons and glial cells possess astrocytes are key regulators of homeostasis, responding to stimuli through upregulation of GFAP and astroglial hypertrophy [24], which could explain the proliferation of inverse GFAP fibers in the tumor and invasion to the pseudo capsule as well as the presence of abundant macrophages, higher Ki-67 values and invasion, aggressiveness, and recurrence as compared to non-apoplectic adenomas [23]. This can be extrapolated

to an association between the hypercoagulable state seen in those diagnosed with COVID-19 and the occurrence of PA in our patients. While much remains to be understood regarding COVID-19, its mechanism of action, and its direct and indirect systemic effects; the aim is to increase awareness of possible neurological complications in this patient population.

Massive cytokine release and thrombogenesis may contribute to this, as they stimulate the accumulation of coagulation factors in plasma and on the surface of endothelial cells, inducing platelet aggregation in the endothelium. It is believed that IL-8 and TNFα may stimulate the release of hyperreactive Von Willebrand factor (VWF), and IL-6 inhibits its division [25]. Levels of all these molecules (VWF, IL-6, IL-8, and TNFα) are elevated in patients with SARS-CoV2 and also VWF has been identified as a marker of endothelial damage in this disease [19, 20]. In our work is observed overexpression of IL6, IL0, and IL7, together with VWF, TNFa+, and TNFk immunoexpression [26].

Extravasation of inflammatory cells with atypical lymphocytes in the perivascular space, polymorphonuclear cells in the lumen in vessels or the endothelial cells layer, and necrosis or hemorrhage areas have been observed. Cytotoxic T lymphocytes could be seen in small amounts [27]. The expression of CD26 is enhanced by T-cell mitogens or antigens. A correlation between CD26 expression and enhanced enzymatic activity was observed after T-cell activation. Primary T lymphocytes induce T-cell apoptosis through extrinsic and intrinsic apoptosis pathways [27]. Activation marker on T cells plays important roles in T-cell functions, including activation, signal transduction, and co-stimulation [20,28]. Inflammation can lead to progression, and invasion of neoplasia via EMT [23]. We only observed CD8+ expression, which corresponds to cytotoxic T cells.

Based on these compliments observed in the PA studied in this pandemic year, we observed that most showed certain changes of necrosis, edema, loose proteinaceous material, necrosis, apoptosis figures, inflammation of extravascular lymphocytes (CD3, CD4, and CD8 +), and intraluminal polymorph nuclear cells and apoptosis in the endothelial cell. Abundant foamy macrophages (CD68 +) and weak positivity for CD163 [26]. With abundant hemosiderin and with an accumulation of a material that was positive for FVIII, DPGF, Hif1a, TNFa, TNFkb, IL6, IL10, and IL17. S-protein-determined inflammasome activation in macrophages isolated from convalescent COVID-19 patients, which correlates with distinct epigenetic and gene expression signatures suggesting innate immune memory after recovery and leads to reprogramming of human macrophages and induces a potent humoral immune response from COVID-19 [27]. Damage to EC with loss, atypia, apoptosis, hyperplasia, intraluminal accumulation, as well as endothelial cells with extravascular proliferation, production of an amorphous material product of hemolysis, and hemosiderin [28,29]. They have been little studied and have not even been described in association with PA, but they are widely described in association with COVID pathology [16,17]. However, it is striking that they were all patients who presented rapidly evolving symptoms and required emergency surgery, in a pandemic period in which no scheduled patients had been operated on. Not all showed a positive PCR and three patients died. COVID-19 RT-PCR testing may produce false-negative results in the initial phase of infection. Unfortunately, we do not have the antibodies to determine if the recent pathology is directly related to COVID-19 in negative patients.

Furthermore, the ultrastructural alterations seen in the endothelial cells of such adenomas may also result from hypoxia deriving from an inadequate blood supply to the tumor. We observed necrosis of the endothelial cells with loss of granules and the presence of abundant mitochondria, observing cells with an oncocytic vs apoptotic appearance, as well as damage to the endothelial cells, their loss, and cellular debris, thinning of the vessel wall.

The pituitary gland has a rich and complex vascular supply, making it more susceptible to haemorrhage. Blood vessels within pituitary adenomas may have low fenestration, incomplete maturation and fragmented basal membranes that contain perivascular spaces filled with red cells and plasma proteins, which could predispose to haemorrhage. Rapid growth of a tumour and increased metabolic activity which outpaces the development of sufficient blood supply to a tumour creates increased demand, which may precipitate PA. Studies have shown ACE-2 receptor expression in gonadotrophs, lactotrophs, somatotrophs and corticotrophs, even though in low levels, as well as TMPRSS2 pituitary expression [30], particularly in hypothalamic regions associated with food intake and metabolic regulation and hypertension.

PA is an endocrine emergency, which commonly presents as hypopituitarism. Prompt diagnosis and treatment can be both life and vision saving. There are a growing number of published case reports postulating a link between COVID-19 and pituitary apoplexy.

Conclusion

Pituitary apoplexy is an endocrine emergency, which commonly presents as hypopituitarism and several endocrinal disturbances. COVID-19 positive patients with PA enter host cells via ACE2 receptor and use the human protease TMPRSS2 as an entry activator, at diverse stages of infection encouraged protein changes characteristic of the endothelial dysfunction and inflammatory response after viral infection, and coagulation process in the pituitary gland. It seems reasonable that further research is done to elucidate any potential relationship between COVID-19 infection and PA. Despite the rarity of this endocrine emergency, the prevalence of COVID-19 suggests that more patients with underlying diagnosed or undiagnosed PTs may be exposed to COVID-19. PA is a medical and surgical emergency due to its association with both hormonal dysregulation, cerebral ischaemia, subarachnoid haemorrhage, brain strokes and death.

Author contributions

All authors made substantial contributions to the conception and design of the work as well as drafted, revised, and approved the version to be published. All authors agree to be accountable for all aspects of the work related to accuracy and integrity.

Conception and coordination of the study: MLTS, AOP; SMJ, JS

Design of ethical issues: MLTS. LCM, SM, DR, JF

Acquisition of clinicopathological and experimental data: DR, MLTS; AOP, SMJ

Analysis and interpretation of data: MLTS. SMJ, AOP, DR,

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Compliance with ethical standards

Conflict of interest

The authors have no conflicts of interest or competing interests to disclose.

Consent

According to international standards or university standards, the patient's written consent has been collected and preserved by the authors. Written informed consent was non-obtained from the patient for publication of this case report. Our institution patients sign an informed consent for every early surgical and laboratory clinic procedure including publication if it is necessary.

Ethical approval

According to international standards, written ethical approval has been collected and preserved by the author (s).

Disclosure

The authors declare that they have no conflict of interest.

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