



Does expression of substance-P in normal appendices confirm the neuroimmune appendicitis or disprove the negative appendectomy?

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ABSTRACT

Introduction: Detection of specific histological alteration in appendices was carried out using immunohistochemical technique involved using antibodies against a neuropeptide substance-P (s-P).

Methods: Fifty patients aged 3-45 years (21 male and 29 female) admitted to Azadi Teaching Hospital, Kerkuk province, Iraq with clear clinical symptoms of appendicitis.

Results: Significant differences ($p \leq 0.034$) was detected at age 11-15 and 16-30 years of patients with clinical suspected appendicitis (CSA) in most patients while right iliac fossa pain (RIFP), vomiting and nausea were predominate clinical symptoms yet non-specific signs of appendicitis. Only 82% appendices appeared inflamed and 18% were normal (without inflammation), were considered as experimental cases. The intensity and location of the s-P expression was classified as either no expression (negative s-P), weak (+) or strong (++). In experimental cases it was strongly expressed in nerve plexus than in acute inflammation of epithelium and muscularis layers while in lamina propria the expression was almost the same between the two groups. The normal appendices with symptoms were found without inflammation yet s-P expression was detectable.

Conclusion: Therefore, the stronger expression of s-P in normal appendices may confirm the concept of neuroimmune appendicitis which disproves the negative term of "appendectomy". Further immunohistochemical studies are on to explore specific histological alteration associated with the pains using other antibodies i.e. vasoactive intestinal polypeptide (VIP) and GAP-43.

Keywords: *Appendicitis, appendectomy, Neuroimmune, neuropeptide, Substance-P expression.*

INTRODUCTION

The sympathetic nerve supply to the appendix, in the visceral peritoneum, reacts to stretch, leading to the early periumbilical pain in appendicitis. Thoraco-somatic sensory nerve fibers respond to inflammation of the parietal peritoneum leading to the classic pain migration to the right iliac fossa (RIF) that covers the basic characteristics of the appendix in its intact status[1]. Previous literatures confirm neural proliferation in the appendix associated with the increased immunologic reaction to the peptide, substance-P (s-P) and to the vasoactive intestinal polypeptide (VIP) in patients with clinical diagnosis of acute appendicitis (AA) without inflammatory reaction[2] and increase of these mediators in the appendix may cause pain on the RIF in the presence of acute appendicitis (AA)[3], meanwhile immunohistochemistry techniques lead to the detection of many neuropeptide in the gut i.e. s-P, VIP and others[4].

The acute appendicitis (AA), a high prevalence disease, requires a rapid and accurate diagnosis to either confirm or exclude perforation which causes abdominal pain, with surgical appendectomy being the standard choice of treatment yet considered a clinical emergency[5]. Although diagnosis of appendicitis is largely clinical but should also be coupled with laboratory confirmation, supplemented by selective focused imaging [6]; meanwhile a delayed diagnosis could lead to complications like perforated appendix, peritonitis, sepsis, increased morbidity and mortality; yet its diagnosis remains challenging[7].

In most patient with AA two important components could attribute to the pathogenesis of AA i.e. obstruction and infection by primary pathogenic event. The latter is believed to be due to luminal obstruction which may result from a variety of causes, i.e. fecalith, lymphoid hyperplasia, foreign bodies and parasite meanwhile bacterial infection is believed to be central to appendix inflammation[8]. Several reports confirmed the importance of bacteria particularly *Escherichia coli*, *Clostridium perfringens* and *Bacteroides* sp. in the pathogenesis of appendicitis which all are normally present in the lumen of the appendix[9]. Cases of appendicitis, have dramatically

increased annually in USA and in UK[10][11]. The function of the appendix in human has been confirmed in 2003 following finding that the immune system supports growth of beneficial (mutualistic) bacteria, as a well-adapted in the mammalian gut and higher in the appendix[12]. There is an overall high density of mucin and sIgA produced by β -cells in the mucosa and outer loose mucus layer of the appendix which is a pro-microbiotic environment to support its function as "safe house"[13]. Appendix does also perform an immunological function as the lymphoid tissues in the appendix develop within the first year of life[14][15]. During teens some atrophy is seen, but the appendix continues its immunological function throughout life, albeit with gradually declining activity[16]. Appendix acts like tonsil to guard the upper alimentary tract from bacteria i.e. it guards the small intestine from bacteria present in the large intestine[17]. Moreover, the appendix is capable of producing mesenchymal stem cells during both infancy and at older ages, which could develop into osteoblasts, lipoblasts and myoblasts, depending on the stimulation, enabling these stem cells to bowel repair throughout life[18].

For over 100 years of study, yet there is no single explanation for all causes of appendicitis; however, there are a few hypotheses i.e. classic hypothesis, which postulates that the obstruction of appendiceal lumen by either a fecalith or lymphoid hyperplasia (11-52%)[19], and it is believed to be a primary pathogenic event in most patients with acute appendicitis[8]. Lymphoid hyperplasia, with an unknown cause, has been suggested to be the underlying cause of purulent appendicitis at the absence of fecalith. Meanwhile it is more common in non-inflamed appendicitis (NIA) than in AA albeit other causes of luminal obstruction (LO) may also be possible [20][21]. Alternative hypothesis for the etiology of appendicitis is based on the concept that either bacterial or viral enteric infection leads to mucosal ulcerative of the appendix (75%) and subsequent bacterial invasion from the normal colonic flora[9]. The etiology may expand to include either hygiene theory i.e. changes in sanitation tied to the industrial revolution[22]; or on seasonal variance i.e. during the warm months of the year in incidence of acute appendicitis (AA)[23]; or in rainy season and high humidity levels could increase[24].

The mechanism of occurrence accompany obstruction and continuous mucus secretion lead to increased intraluminal pressure which can reach 50-65 mm/Hg but once luminal pressure exceeds 85 mm/Hg, thrombosis of the venules that drain the appendix starts cause arteriolar inflow, vascular congestion and engorgement of the appendix which become manifest leading to lymphatic and venous drainages are impaired and ischemia develops[8]. Mucosa becomes hypoxic and begins to ulcerate, resulting in a compromise of the mucosal barrier, leading to invasion of the appendiceal wall by intraluminal bacteria. As a result, visceral afferent nerve fibers that enter the spinal cord at T8-T10 are stimulated, causing referred epi-gastric and peri-umbilical pain represented by the correspondent dermatomes. At this stage, somatic pain supersedes the early referred pain, and patients usually undergo a shifting on the site of maximal pain to the right lower quadrant (RLQ)[25][26]. The presence of a normal functioning appendiceal mucosa is required for fluid secretion and development of the histologic picture of AA. If this allowed to progress, arterial blood flow is eventually compromised and infarction occurs, resulting in gangrene and perforation, which usually occurs between 24-36 hours. Hence, anorexia, nausea, and vomiting usually follow as the pathophysiology worsens[27][28].

In autopsy pathology, while basic histologic examination of tissue is considered a useful and necessary component yet immunohistochemistry (IHC) may provide a greater insight[29]. Specific molecular markers are characteristic of particular cellular events i.e. proliferation or cell death[30]. Visualizing an antibody-antigen interaction can be accomplished in a number of ways e.g. an antibody is conjugated to an enzyme i.e. peroxidase, that can catalyze a color-producing reaction[31]. Substance P (s-P) is a highly conserved peptide and an undecapeptide, derived from the pre-protachykinin-A gene, which is differentially spliced to form different mRNAs[32]. The s-P is synthesized, mostly by neurons, as a large protein, and is transported to the neuronal terminal endings, where it is enzymatically converted into the active form and stored in vesicles ready for release. The s-P and its cognate receptors are present in neurons, as well as in microglia, endothelial cells, and peripheral immune cells[33] and is widely distributed throughout the central nerve system

(CNS), peripheral nerve system (PNS), and enteric nervous systems (ENS). It is present in dorsal root ganglia (DRG) in primary afferent sensory neurons[34][35]. The s-P is also expressed by some stem cells and progenitor cells[36], including immunomodulatory mesenchymal stem cells (MSC)[37].

There has been a neural proliferation in the appendix, associated with the increase of an immunological reaction for s-P and VIP, in patients with clinical diagnosis of AA without inflammatory reaction. Increase on the staining of nerve fibers for protein gene product 9.5 (PGP 9.5) in the mucosa of non-inflamed appendices (NIA), as well as the presence of both s-P and VIP may denote the cause of the pain on the right flank (RF) in the presence or not of an inflamed appendix[38]. In the basal mucosa, the neurogenic vasodilatation is mediated not only by s-P, but also by the release of VIP showed that the distribution of the VIP and its expression are changed in the inflammatory intestinal diseases[39]. Accordingly, the changes in the peptidergic innervations in AA may be related to localized pain[40]. In an immunohistochemical examination of appendectomy, which were NIA, a proportion exhibited an excess of neurotransmitters i.e. s-P and VIP had lead the authors to suggest the concept of neuroimmune appendicitis[3], although this has not been confirmed by others[41]. The latter followed speculation that removal of the appendix might lead to an improvement of symptoms in these individuals, with a recent report claiming that an appendectomy can lead to a reduction in right lower quadrant pain (RLQP) in a selected group of patients[42]. The s-P exerts a wide range of both physiological and pathological effects, particularly, in nociception and neurogenic inflammation [43] [44][45] primarily mediated by the NK1R receptor; however, the diverse expression of NK1R on various non-neuronal cell types[46] suggests other functions in addition to its role in pain, including growth-promoting effects on smooth muscle cells[47], skin fibroblasts[48], and synoviocytes[49]; regulating angiogenesis and vasodilation by controlling the release of nitric oxide[50]. Elevated levels of s-P and upregulated NK1R expression are seen in the rectum and colon of patients with inflammatory bowel disease (IBD) and correlate well with disease activity[51].

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The s-P is a part of an immune-regulatory mechanism that amplifies inflammation at intestinal mucosal surfaces in acute phase of IBD[52], meanwhile it also is present at sites of inflammation, and inflammation can enhance its expression[53]. All above data suggest the involvement of s-P in appendicitis. The "neuroimmune appendicitis" is a new concept for normal appendix cases of patients with clinically diagnosed appendicitis, introduced by Di Sebastiano and co-workers[3]. This research has been designed to investigate the histological change accompanied in the appendix and to verify the expression of substance-P in histologically normal appendix in comparison with positive control.

MATERIALS AND METHODS

This study involved 50 samples of appendix (21 males and 29 females) subjected to appendectomy where appendices are collected directly from operation theatre at Azadi-Teaching Hospital at Kerkuk Province, Iraq. Specimens were placed in 0.9% saline to wash out blood prior transferring them into 10% formalin in 0.9% saline for fixation. Following 24hrs postfixation specimens were processed for routine tissue technique at the room temperature of the laboratory[9]. Tissue were stained with either hematoxyline and Eosin (H&E) or processed for immunohistochemistry technique using antibodies raised against substance-P at Liverpool Veterinary laboratories, UK by Ms Valarie Tiliston. Followed by double examination of all appendicitis H&E slides by histopathologist (Dr. Zana T. Abdulrahman) at Azadi hospital to confirm the results.

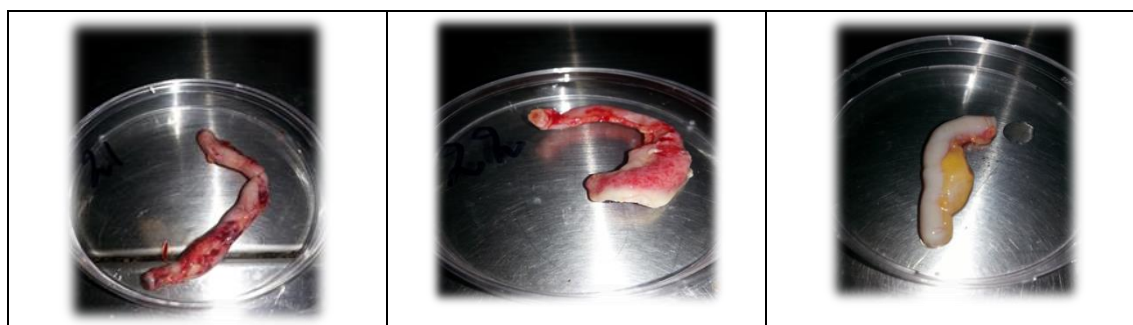


FIG. 1: Three different appendix samples in Petri dishes with occasional hematoma, blocked by fecalith and with attached adipose tissue, respectively. Specimens were cut into smaller pieces for histological processing.

A comparison of expression of s-P was carried out of positive control acute appendicitis (AA) with experimental group (histologically normal appendices). The reading of slides was double checked by another pathologist, Dr. Tamara Al-Mufty according to subjective study involved intensity and the location of the s-P staining i.e. (+1) weak and (+2) strong or non-staining as negative s-P. Classification of appendix walls in terms of development of neutrophils was followed according to[54].

RESULTS

The overall age of patients ranged between 3-45 year with majority at age groups, 11-15 and 21-

30 year. Histologically, the individuals with normal appendices were occasionally found within five age groups (11-15; 16-20; 21-25, 26-30, 41-45 years) while other 4 groups had appendicitis (Fig. 2A). The most inflamed appendices cases were in age group 11-15; 21-25; and 26-30 years old. There was a significant difference ($p \leq 0.034$) between age group and patients with clinically suspected appendicitis. The male patients were 21(42%) from the total clinically diagnosed suspected appendicitis had all confirmed histologically to be appendicitis while only 20 females out of 29(58%) were appendicitis. There is a significant ($p \leq 0.005$) difference between gender and patients with clinically suspected appendicitis (Table-1).

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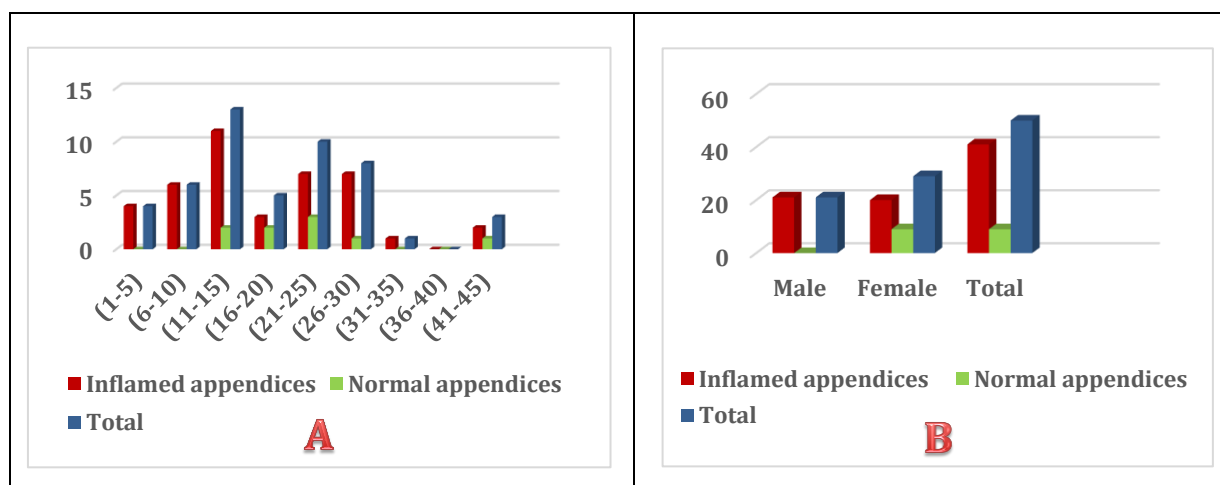


FIG.2 A & B: (A). The distribution of appendicitis amongst the age groups of the patients. (B). Types of histologically diagnosed appendix specimens in both genders.

TABLE 1: Classification of appendicitis according to histological diagnosis, gender with significant differences (* $p \leq 0.005$).

Gender	Inflamed appendices		Normal appendices		Total No.	%
	No.	%	No.	%		
Male	21	100	0	0	21	42.0
Female	20	69*	9	31*	29	58.0
Total	41	82	9	50	50	100
Chi-Square	$p \leq 0.005$					

The general symptoms of the patients diagnosed by a physician prior to appendectomy were almost 4 main symptoms e.g. vomiting (56%), nausea (28%), anorexia (16%) and diarrhea (6%). Although these symptoms appeared higher in inflamed appendix in comparison with normal appendices, the differences, yet, were insignificant. The insignificant differences were also detectable when compared symptoms with genders. Similarly, both anorexia and diarrhea symptoms showed much higher reading in female in comparison with males, yet the difference was insignificant; meanwhile whenever the nausea and anorexia symptoms gathered in patients, they still showed higher in females than in males. In some cases, there were some common symptoms between the patients i.e. vomiting with nausea (12%); vomiting,

nausea and anorexia (6%); nausea and anorexia (10%) and vomiting with diarrhea (4%).

All patients had suffered abdominal pains prior admitting hospital for surgery. The pain lasted from 15 hrs to 5 days while in only 2% cases the pain re-started at following intervals for 3 months. Three types of pain were recognizable i.e. right iliac pain (RIFP) [88%], right lower abdominal pain (RLAP) 8% and abdominal pain (AP) 4% where the pain started at the umbilical cord area and migrated to the RIF [Table. 2]. Almost all the pains displayed severe colic in these patients during admission to the hospital. The duration of the pain ranged between <1 day to 5 days in the RIF and between <1 day to a few days in the RLAP while in abdominal pain it ranged from 1-2 days (Table-3).

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TABLE 2: Details of histologically diagnosed appendix specimens types in both genders.

Gender	Male	Female	
Type of appendicitis	No.& (%)	No. & %	Total & (%)
Acute appendicitis (AA)	56.0(28)	46.4 (13)	53.6 (15)
Acute suppurative appendicitis (ASA)	14.0(7)	57.1(4)	42.9 (3)
Acute appendicitis/peri-appendicitis (AAP)	6.0(3)	33.3(1)	66.7 (2)
Acute gangrenous appendicitis (AGA)	2.0(1)	100(1)	0.0 (0)
Resolving appendicitis (RA)	4.0(2)	100(2)	0.0 (0)
Normal	18.0(9)	100(9)	0.0 (0)
Total	100(50)	58.0(29)	42.0(21)

TABLE 3: Three recognizable types of pain with percentage among patients: (AP), abdominal pain; (RLAP) right lower abdominal pain and (RIFP), right iliac fossa pain.

Duration & Age Pain type	Age (years)	1> Day (%)	1 Day (%)	2 Day (%)	3 Day (%)	4 Day (%)	5 Day (%)	Total (%)
AP	25-28	-	2	2	-	-	-	4
RLAP	5-20	2	-	4	2	-	-	8
RIFP	3-45	-	64	14	6	2	2	88

Normal appendices (NA) made 9/50 and 41 were inflamed appendices. Only 5/9 patients of normal appendices had fecalith while the rest were with no fecalith. Nearly 44% inflamed appendices had fecalith in and 19 without. However; the percentage of fecaliths included 30% acute appendicitis (AA); 12% with acute superlative appendicitis (ASA); only 2% acute appendicitis and acute peri-appendicitis (APA). No fecalith was found in Resolving and Acute gangrenous appendicitis. The lymphatic hyperplasia and infiltration of most types of white blood cells (WBCs) dominated by Neutrophils of the appendicitis of all 50 cases with some other histological disorders i.e. vacuolation, fibrosis, and erythrocytes (RBC) in two samples. Moreover, necrosis of the mucosa, development of Hassal-like corpuscles in the lymphatic nodules, and closed lumen of the appendix were

detectable in other cases. The alteration in tissues of the appendix walls was classifiable into five types (a). Neutrophilic wall of AA; (b). Aggregate of neutrophilic cells and cellular debris in wall of ASA; (c). Hemorrhagic in wall AGA; (d). Fibrosis with chronic inflammatory cells RA; and (e). Acute inflammatory cells in serosa AAP (Fig 3A-3F). In general the majority incidence (56%) of appendicitis were either AA or ASA which appeared almost equally 50%±5 between male and female patients. Other three causes i.e. acute appendicitis and periappendicitis, acute gangrenous appendicitis and resolving appendicitis showed lesser proportion 6%, 2% and 4%, respectively. However, in only 18% of normal cases the appendix tissues appeared intact; however, almost all normal cases were found in females (Table-3).

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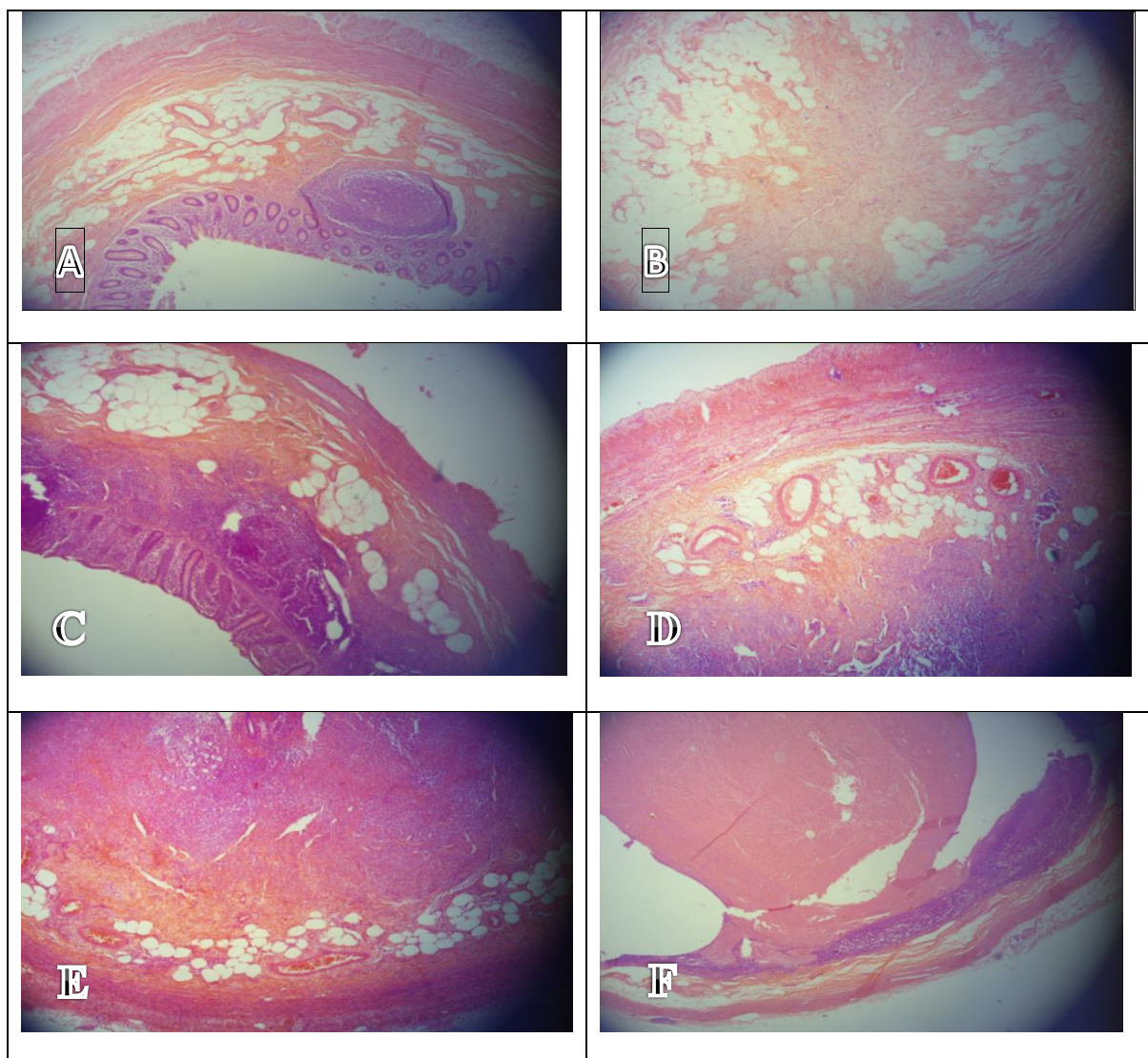


FIG. 3A-B: [A]. CS in normal appendix with various types of acute appendicitis (AA): [B]. Resolving appendicitis (closed lumen); [C]. Lymphatic hyperplasia; [D]. Dilation of blood vessels with diffuse lymphatic cells in the submucosa [E]. Hemorrhage, infiltration of RBC and WBC and [F]. Fibrosis in the lumen (H&E,50X).

Immunohistochemistry(IHC)

Four out of 10 acute inflammation showed no expression of s-P in the epithelium while other 6 had weak staining in comparison with experimental group. In 9 samples, only 4 showed weak staining meanwhile other 5 showed strong staining with staining detected in muscularis layer. Amongst the layers of the appendix, the lamina propria, in positive control of the AA all showed weak staining in comparison with experimental group with only a single sample showed no-staining while other 8 had weak staining. In experimental group the expression of

the s-P was even stronger i.e. 4 weak staining and 5 strong staining, with a single sample showed strong staining. Nerve plexus appeared equally distributed in weak and strong in positive control. However, in experimental group the expression of s-P of the nerve plexus appeared weak in a single sample while other 8 showed strong staining (Fig.4).

In positive control (inflamed appendices) the intensity of s-P of nerve components varied among the layers (epithelium, lamina propria, muscularis and adventitia) from weak staining (+1) to strong staining (+2).

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However, none of the epithelium layer of the four cases of AA the s-P was ever expressed in (Table 5). The intensity of staining of s-P was stronger in experimental group especially in nerve plexuses with just in one case the s-P with a weak staining. However, in five cases of

experimental group patients had no symptoms prior appendectomy. In most cases of infection, the s-P has been less expressed in lamina propria than in epithelia, muscularis and adventitia (Fig. 5 and Table-6).

TABLE 5: Distribution and intensity of s-P in 2 study groups: positive control (AA) and experimental group (normal appendix). Note the expression of s-P is higher in experimental group than in acute appendicitis.

Study groups	Positive control (Acute Appendicitis, n=10)			Experimental group (normal appendices, n=9)		
	None	+1	+2	None	+1	+2
Staining intensity	4	6	-	-	4	5
Epithelium	4	6	-	-	4	5
Lamina propria	-	10	-	1	8	-
Muscularis	-	9	1	-	4	5
Nerve plexus	-	5	5	-	1	8

TABLE 6: The intensity of s-P staining in experimental group with symptoms and the type and duration of pain.

Experimental group (normal appendix) [n=9]			Symptoms	Type of pain	Pain duration (day)
Staining intensity		No.			
Epithelium	+1	1	No	RLAP	2
Lamina propria	None				
Muscularis	+1				
Nerve plexus	+1				
Epithelium	+1	2	No	RIFP	1
Lamina propria	+1				
Muscularis	+1		Vomiting & Nausea	RIFP	1
Nerve plexus	+2				
Epithelium	+1	1	None	-	-
Lamina propria	+1			-	-
Muscularis	+2			-	-
Nerve plexus	+2			-	-
Epithelium	+2	1	Vomiting, Nausea and Anorexia	RIFP	1
Lamina propria	+1				
Muscularis	+1				
Nerve plexus	+2				
Epithelium	+2	4	Vomiting	RIFP	1
Lamina propria	+1		Vomiting	RLAP	2
Muscularis	+2		No symptoms	RIFP	2
Nerve plexus	+2		No symptoms	RIFP	1

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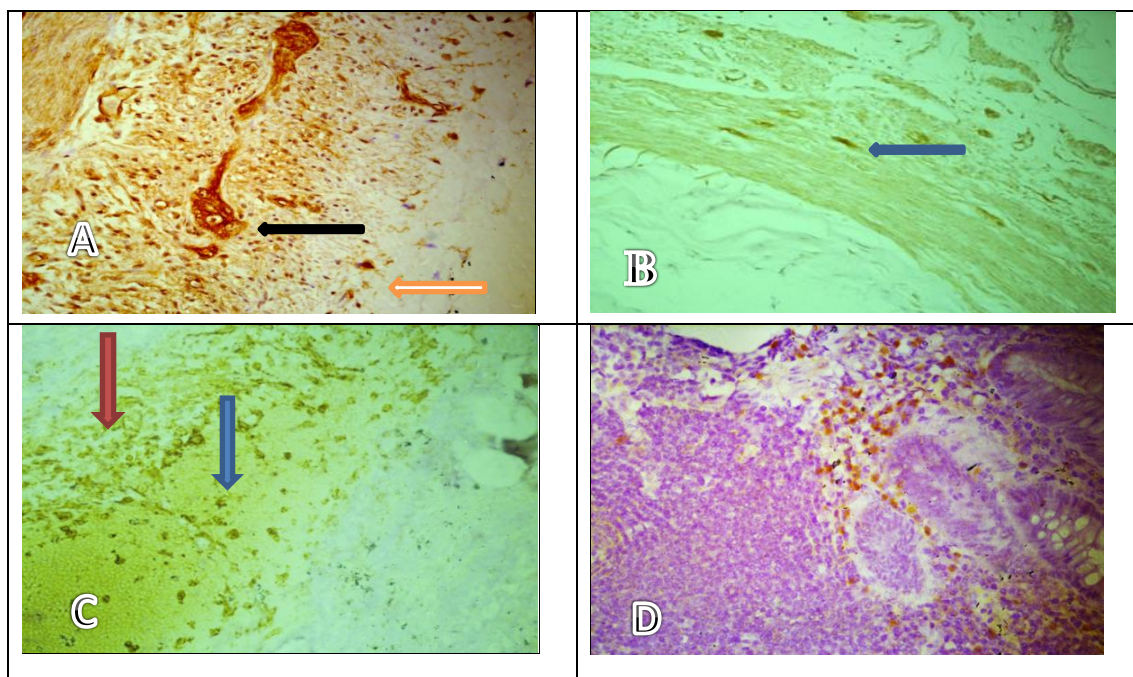


FIG. 4: [4A] s-P+ neurons (white arrows) with many fibroblasts (black arrow) expressed in their whole cytoplasm in the lamina propria of the appendix, positive control (IHC, x400). (Fig.4B) Expression of some s-P+ nerve cells within the adventitia of the appendix (blue arrows), positive control (IHC, x200); [4C]; and expression of some s-P+ cells (blue arrow) within the hematoma patches of RBCs in the submucosa layer of the appendix (red arrow). (4D) Positive control [IHC, x400].

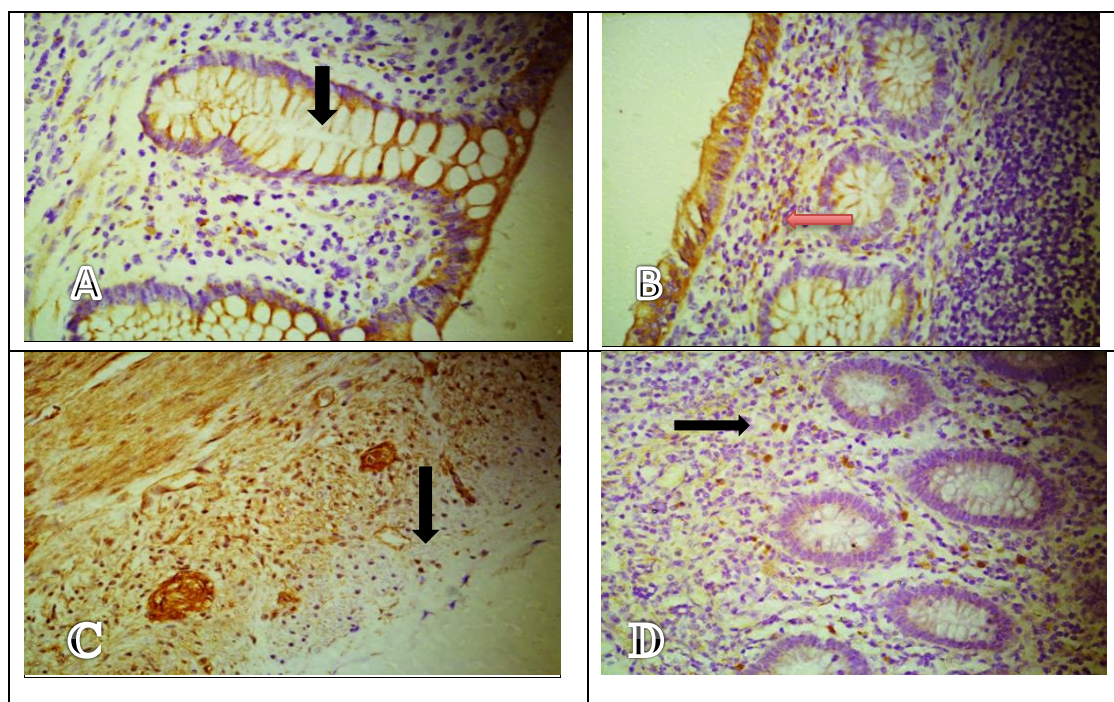


FIG.5: [5A] The s-P+ (black arrow) is expressed mostly in the epithelial cells around goblet cells but evenly distributed in the lamina propria in the crypt (black arrow) [Positive control (x400); (5B) The s-P+ epithelial tissue rich in goblet cells is expressed in their whole cytoplasm as well as in the lamina propria (red arrow) of the appendix(x400). (5C) The expression of s-P in the adventitia (black arrow) (x400). In Fig.5D the positive processes of substance-P cells (black arrows) in clear in the lamina propria of Experimental group (x400).

DISCUSSION

Fifty patients with clinical symptoms of appendicitis, with right lower abdominal pain (RLAP) admitted to hospital were diagnosed, by physicians, as acute appendicitis (AA). Medical diagnosis of appendicitis is largely based on both person's symptoms, but whenever the diagnosis is unclear, a close observation, medical imaging, and laboratory tests deem helpful [55][56]. The two most common imaging tests used are an ultrasound and computed tomography (CT scan) which represents more accurate than ultrasound in detecting AA[57]. Nevertheless, ultrasound as the first imaging test in children and pregnant women is preferred over CT scan because of the risks associated with radiation exposure from CT scans [55]. The CT scan, is used in Iraqi hospitals upon admission of patients into the hospital complaining abdominal pain. Appendectomy is carried out albeit further tests may be necessary by health authorities to assure appendicitis prior operation. In 62% appendectomy cases the pain lasted for 24 hours before operation which indicates emergency incidence; hence, acute appendicitis (AA). Cases lasts >24 hours might refer to either non-acute cases or the pains are ameliorable with some painkillers subject to appendectomy pending the outcome of clinical analysis. Considering all possible etiology of appendicitis, a wide range of age of patients ranged (3-45 years old) had undergone appendectomy. This could likely be interpreted either individuals >46 years old are more resistant to infection, more curious to their food quality consumed than younger ages or perhaps have efficient immune system. This result is in agreement with a study in Taiwan [58], but can occur at any age [59]. Therefore, it is feasible that appendicitis is more common in younger than in elder people. The appendicitis is caused mostly by a blockage of the hollow portion of the appendix due to a calcified "stone" made of feces [60]. Inflamed lymphoid tissue from a viral infection, parasites, gallstone, or tumors may also cause the blockage etiology[61]. The blockage would therefore, lead to increased pressures in the appendix, decreased blood flow to the tissues of the appendix, and bacterial growth inside the appendix causing inflammation [56]. In Iraq, fecalith cases have been common in the present research.

The higher proportion of females than males found in this study is in agreement with a recent

study [62] but contradicts another [63]. Prior appendectomy, all patients suffered from abdominal pains but in majority of patients this pain migrated especially to right iliac fossa (RIF) or sometimes to right lower of abdominal (RLA). This may reveal the misdiagnosis chance of appendicitis cases in women that might have misinterpreted with other disorders i.e. ovarian cystitis and other inflammation with different causes. Other symptoms accompanied some patients i.e. vomiting, nausea, anorexia and diarrhea (56%, 28%,16%, 6%), respectively; varied among patients themselves subject to their complaints. These symptoms appeared higher in inflamed appendices in comparison with normal appendices leading to conclude wrong diagnosis or not accurate enough to rely on as appendicitis. External symptoms-like signs of appendicitis i.e. LAP and LIFP were prominent in almost all incidents of appendicitis admitted to the hospital. Fecalith was found in both genders and made up 54% of appendices as a main cause of appendicitis. Perhaps the second etiology might involve uncommon causes e.g. parasites, undigested plant or fruit residues, trauma and foreign bodies. Other authors have concluded that the prevalence of fecalith was more prevalent in pediatric than in adult[64]. Appendicitis in children is closely associated with lymphoid hyperplasia and may be often due to viral causes [65]. Despite low number of patients involved in this study yet, fecalith could be a common cause of obstruction in comparison with other cause mentioned above.

The routine histological staining (H&E) showed that nine out of fifty appendectomy appeared normal (no histopathological alterations), were chosen as *experimental group* to compare the substance-P expression intensity with positive control (AA). When compared with a normal pathology, reports in a patient with RLQ abdominal pain suggestive of AA the physicians often labels the surgical procedure as "Negative appendectomy" and attribute the case of abdominal pain to a nonspecific temporary cause. However, the histopathological examinations revealed that 82% of them were genuine cases of appendicitis and 18% was normal (NA). Our initial result is in agreement with two similar studies with close proportion of normal appendices made only 17% [66] and 11% [67], respectively.

The normal incidents of appendectomy could have hereby been considered either prejudgment or clinically misdiagnosing of appendicitis. The inflammation accompanied with reduced blood flow to the appendix and distention of the appendix could cause tissue injury and tissue death, when left untreated, then appendix may burst, releasing bacteria into the abdominal cavity, leading to further complications [57]. The high rates of NA appendectomy in Iraq, might not absolutely be a wrongdoing but could well be acceptable in an attempt to eliminate the source of pain or any possible subsequent complications of appendicitis i.e. perforation, peritonitis, abscess formation and sepsis which the victim can undergo [68]. Such cases might be called an "acute appendicitis" where its standard treatment is surgical removal of the appendix [56][61]. By appendectomy the risk of side effects or death associated with rupture of the appendix will be decreased or eliminated [69].

The two main histological changes during appendicitis have been: (1). the neutrophilic infiltrate of the muscularis layer which is the definitive diagnosis based on pathology and (2). Peri-appendicitis, inflammation of tissues around the appendix, could also be often found in conjunction with other abdominal pathology [70]. These tissue alterations were noticed in our samples in addition to others e.g. lymphatic hyperplasia, local disintegration of mucosal layer.

Markers of inflammation i.e. cell adhesion molecules and cytokines may be detected by immunohistochemistry techniques before the appearance of an inflammatory infiltrate [71][72]. Perhaps testing of histologically normal appendices for the expression of these markers of inflammation in patients with a clinically diagnosed AA should reveal an "early inflammation" and potentially transform a "negative appendectomy" into a "valid" one. A growing body of evidence suggests another etiology of pain from appendiceal origin in patients with a "negative appendectomy" is the "neuroimmune appendicitis" introduced by Di Sebastiano and co-workers [41].

Only 10 samples of AA were chosen as positive control (not gangrenous, suppurative,

periappendicitis, nor resolving appendicitis) which were compared with nine cases of experimental group (normal appendix cases). The two groups classification i.e. positive control (which confirmed clinically and histologically acute appendicitis) and the experimental group (confirmed to be clinically acute appendicitis but normal histologically) according to [40]. The present result is concomitant with another work where the area of s-P immunoreactivity was proportionately larger in the non-acute appendicitis than in the AA or in the controls[3]. Their concept of "neuroimmune appendicitis" for histologically normal appendix cases with (RLAP) experienced by some patients has been supported by Bouchard and co-workers. The clinical implications of "neuroimmune appendicitis" implies that patients with a "negative appendectomy" were not erroneously performed but rather was indicative. The latter may disprove, in part, the principle of the "Negative appendectomy[40]. However, whether the stronger expression of s-P in a normal appendices does confirm the "Neuroimmune appendicitis" terms for histological normal appendices or disprove the "Negative appendectomy" terms needs confirmation by further studies i.e. expression of neuropeptide (s-P and VIP) in patients with clinically diagnosed as appendicitis but histologically was normal in comparison with inflamed cases of appendicitis. Perhaps more normal specimens of appendices with symptoms of appendicitis would be beneficiary for rather accurate conclusion.

CONCLUSION

Appendicitis is more common in younger than in elder ages with significant differences between genders and insignificant variations between symptoms and patients with clinically suspected appendicitis. The AA is more common than suppurative, peri-appendicitis, resolving and gangrenous appendicitis. The expression of s-P staining in experimental group was strong in nerve plexus of epithelium and muscularis layers than in positive control meanwhile the expression of s-P was almost same between in lamina propria of the two groups.

Disclosure of conflict of interest and Ethical code of conduct

The authors declare there is no conflict of interest and the work presented in this article is original. For the purpose of confidentiality, no names of the patients ever are disclosed for the public. This research has been fully funded by the authors themselves.

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