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Research Article

Geographical Variation, Gender and Interleukin 8 Relationships with Appendicitis in Residents of Niger Delta Region of Nigeria

Beredugo S.¹, Oboma Y.I.¹, Ilegbedion I.G.¹, Sadjere O.D.², Okara N.P.³

^{1,2,3}*Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.*

²*Nigeria Airforce Medical Centre, Yenagoa, Bayelsa State.*

³*Department of Medical Laboratory Science, Faculty of Sciences, River State University, Port Harcourt*

ABSTRACT

Appendicitis is characterized by inflammation of the vermiform appendix accompanied by systemic inflammatory response in humans. In most cases, emergency surgical intervention is required because it is the leading general surgical cause of death. Nevertheless, accurate diagnosis is still a challenge because of the variation in its manifestations. This work evaluated the role of gender, seasonal variation and the immunohistochemical expression of IL-8 cytokine in appendicitis patients attending Federal Medical Centre (FMC), Yenagoa. A total of 313 appendicitis patients were recruited for the study from January 2015 to December 2020. Results shows that seasonal variation affect appendicitis outcome with statistically significant increase in number of cases recorded in summer compared to winter. Females presented with more cases than males among subjects studied, accounting for 56.9% (n = 178) and males 43.1% (n=135) of survey subjects. Interleukin 8 was markedly expressed in the appendicitis tissue across age, gender and seasons. The study observed the clinical relevance of interleukin 8 in the prediction or diagnosis of appendicitis within the population studied. Risk factors should be monitored more in summer to reduce the occurrence of appendicitis in human population.

Keywords: *Appendicitis, Immunohistochemistry, FFPE*

*Author for correspondence: Email: idslyber@gmail.com; Tel: +234-8067520637

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INTRODUCTION

The vermiform appendix extends from the caecum, immediately distal to the ileocaecal junction of the human large intestine, and measures roughly 2–20 cm long (average 9 cm in adults) and 0.5–1 cm wide (Marie, 2012). Inflammation of the vermiform appendix accompanied by a systemic inflammatory response in human is known as appendicitis, a medical and surgical disorder. About 7% of people experience appendicitis, a prevalent disorder requiring urgent surgery (Sevgi *et al.*, 2015). Before puberty, appendicitis occurs equally frequently across all genders. Appendicitis with a perforation (perforated appendix) is the most common general surgical cause of mortality worldwide (Nischal, 2014). Appendicitis can affect anyone at any age, but it's uncommon before the age of five. It peaks during adolescence. Age, gender, socioeconomic level, dietary habits, and genetic variables are all linked to the development of acute appendicitis (Stein *et al.*, 2012; Yildiz *et al.*, 2013). Although the exact etiology of appendicitis is not yet known, it is thought to result from a variety of pathogenic pathways,

with obstruction of the lumen being the primary factor in acute appendicitis (Pham *et al.*, 2016). The obstruction causes bacterial proliferation, which will raise luminal wall pressure as long as mucus is still being secreted. Due to the decreased lymphatic and venous drainage, mucosal ischemia also occurs. Together, these factors encourage a localized inflammatory process that may lead to gangrene and perforation. The obstruction could be brought on by tumors, parasites, lymphoid hyperplasia or fecaliths. The most typical cause of appendiceal blockage is fecalith. Luminal occlusion with fecaloid is the most frequently recognized theory of disease development (Marie, 2012).

One of the most frequent differential diagnoses in a surgical emergency department is acute appendicitis. However, due to the variety of its symptoms, correct diagnosis is still difficult. Numerous signs and symptoms of appendicitis, including nausea, weight loss, and right-sided iliac fossa pain have only been given minimal or of no diagnostic significance (Afuwape *et al.*, 2018). In cases where appendicitis is suspected, concurrent examination of clinical and laboratory findings have a better diagnostic value. As a

result, adding laboratory tests can help to strengthen the clinical diagnosis of appendicitis.

The incidence rates of negative appendectomy and perforated appendicitis have not been found to significantly decrease, even though several imaging modalities help in its identification. Inflammatory and immune response variables or markers are significant in the inflammatory appendix. These elements highlight how crucial cytokines are in deciding how appendicitis develops. To coordinate a powerful, self-contained, yet coordinated immune response to the target antigen, immune cells in the body interact with one another through molecules called cytokines (Daneshmandi *et al.*, 2009). Due to the fact that appendiceal infection causes both regional and systemic reactions, as well as conventional therapy enables assessment of regional (peritoneal) and systemic (blood) cytokine concentrations, the inflammatory response in appendicitis can be quantified and characterized (Marlon *et al.*, 2018). It is against this background, the researchers wish to evaluate the immunohistochemical expression of IL-8 among appendicitis patients attending Federal Medical Centre, Yenagoa, Bayelsa State.

MATERIALS AND METHODS

Study Area: The study was carried out in Federal Medical Centre, Yenagoa, Bayelsa State. It is a tertiary health institution and serves as a referral centre within and outside the state. It is a 500-bed hospital that serves as a training centre for both Medical and Allied Medical Science students of the Niger Delta University.

Study Population: The study was carried out among 313 appendicitis patients attending Federal Medical Centre (FMC), Yenagoa, Bayelsa State. Data was collected from January 2015 to December 2020. Formalin fixed paraffin embedded (FFPE) archived appendix tissue blocks were analysed.

Statistical Analysis: Data generated was analysed using the Statistical package for the social sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; version 23.0. Odds ratios and 95% confidence intervals (CIs) for relative risks was calculated. P<0.05 was considered statistically significant, and all reported P-values were two-tailed.

Hematoxylin and Eosin Staining: Appendix tissue sections were dewaxed in xylene and hydrated in descending grades of alcohol. They were stained in Erhlich’s heamtoxylin for 15 minutes, rinsed immediately in distilled water, differentiated in 1% acid alcohol for 1 minute, rinsed in distilled water, blued in Scott’s tap water for 10 minutes, counterstained with 1% eosin, and rinsed in distilled water. Sections were dehydrated in ascending grades of alcohol, cleared in xylene, mounted with DPX and viewed under the microscope using x10 and x40 objectives respectively.

Immunohistochemistry Procedure: Appendix tissues sections were dewaxed by heat, hydrated with descending grades of alcohol and incubated with an enzyme, HIER for antigen retrieval and immediately placed in deionized water.

The sections were flooded with 3% hydrogen peroxide in phosphate buffer (PBS) to block endogenous peroxidase and rinsed in buffer to avoid background blocking. They were incubated with primary antiserum (rabbit antiserum) and rinsed immediately with buffer, incubated with secondary reagent (biotinylated goat anti-rabbit immunoglobulins) and rinsed with buffer. The sections were then incubated with ABC complex and rinsed with buffer, and also incubated with developing reagents (DAB and hydrogen peroxide) to enable detection of immune reaction. Finally, sections were rinsed with deionized water, counter stained with Mayer’s haematoxylin, dehydrated, cover-slipped for observation under the microscope.

Table 1: Demographic statistics of participants.

Age (Yrs.)	Gender		Total
	Male (%)	Female (%)	
0 – 9	12 (8.9)	4 (2.2)	16(5.1)
10 – 19	33 (24.4)	63 (35.4)	96(30.7)
20 – 29	25 (18.5)	44 (24.7)	69(22.0)
30 - 39	24 (17.8)	35 (19.7)	59(18.8)
40 – 49	14 (10.4)	20 (11.3)	34(10.9)
≥50 YRS	27 (20.0)	12 (6.7)	39(12.5)
TOTAL	135 (100)	178 (100)	313(100)

Ethical Approval: Ethical approval for this study was given by the Ethics Committee or Institutional Review Board of the Federal Medical Centre, Yenagoa, Bayelsa State.

RESULTS

Shown in Table 1 is the demographic data of appendicitis patients for this study. Out of 313 patients, 178 (56.9%) were females while 135 (43.1%) were males. There were more subjects in the age range of 10-19 years (63 subjects at a 35.4% occurrence for females and 33 subjects, 24.4% for males).

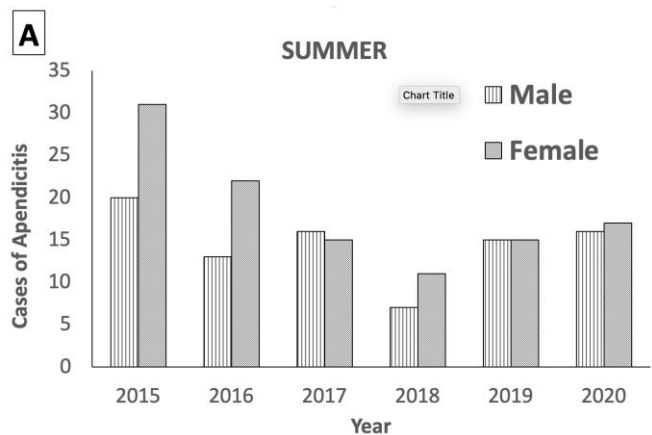


Figure 1a Cases of Appendicitis in study area during summer of years 2015 to 2020.

Figures 1a and 1b show seasonal variation effect on the cases studied. A significant increase was recorded in number of cases among males in 2015 in summer compared to winter

and was statistically significant $p \leq 0.05$. A non-statistically significant reduction in cases recorded in winter was observed in both gender between 2017 and 2020. While a statistically non-significant increase was observed in 2018 in both gender and in 2019 in females only (15 in summer to 16 in winter; $p=0.328$) females also showed a significant decrease ($p=0.001$) from 22 to 5 subjects in 2016.

Plate 1 is a photomicrograph showing reactive lymphoid hyperplasia and fibrotic appendicitis with peritonitis of the appendix stained with haematoxylin and eosin and IL-8 antibody. White arrow showing brownish staining indicates areas of positive expression of IL-8 antibodies. Plate 2 is a photomicrograph showing follicular hyperplasia of the appendix and mucocele appendicitis respectively, both showing marked positive expression of IL-8 antibodies as indicated by the arrow; while plate 3, is a photomicrograph showing necrotizing appendicitis with positive expression of IL-8 antibodies.

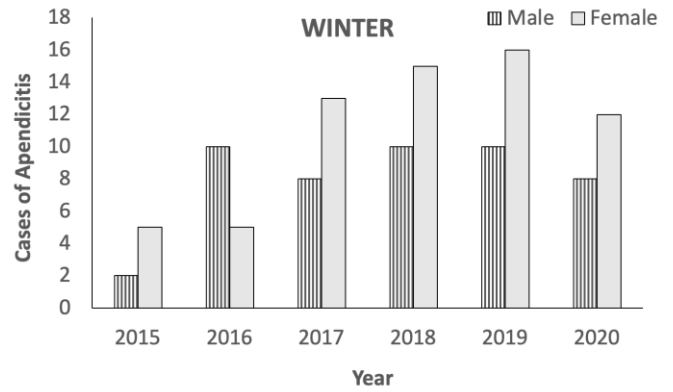


Figure 1b
Cases of Appendicitis in study area during winter of years 2015 to 2020.

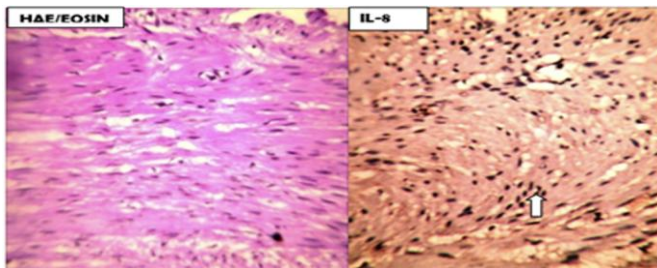


Plate 1a
Reactive lymphoid hyperplasia of the appendix showing mild positive expression of interleukin-8 (IL-8) antibodies). (x400 magnification).

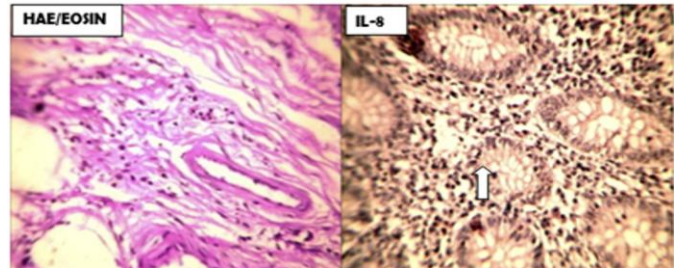


Plate 1b
Fibrotic appendicitis with peritonitis showing positive expression for interleukin-8 (IL-8) antibodies). (x400 magnification).

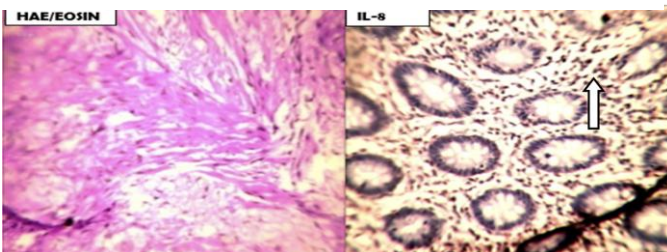


Plate 2a
Follicular hyperplasia of the appendix showing positive expression of interleukin-8 (IL-8) antibodies). (x400 magnification).

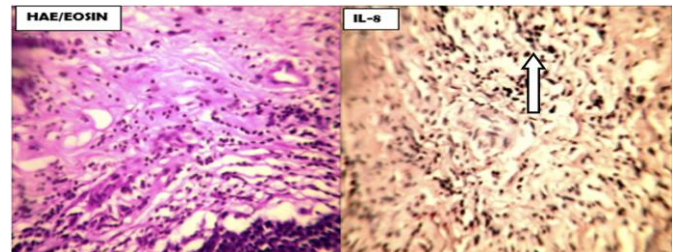


Plate 2b
Mucocele appendicitis showing positive expression of interleukin-8 (IL-8) antibodies). (x400 magnification).

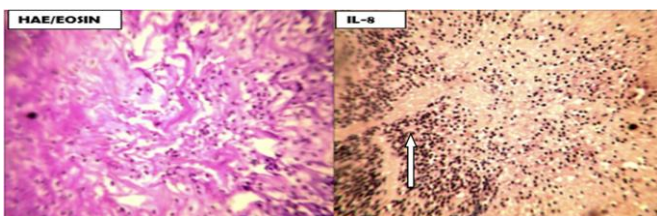


Plate 3
Necrotising appendicitis showing positive expression of interleukin-8 (IL-8) antibodies)

DISCUSSION

Appendicitis is an inflammation of the vermiform appendix in human beings; and it is found to be one of the most common medico-surgical condition that requires emergency surgical intervention (Sevgi *et al.*, 2015). Studies claim that it is

difficult to make a clinical judgment between conservative and surgical treatment for acute appendicitis, particularly in children. Acute appendicitis is categorized as one of the most frequently misdiagnosed illnesses in pediatric surgery as a result of the substantial number of misdiagnosis or appendiceal perforations (Kaiser *et al.*, 2002).

According to Stankovice *et al.* (2019), the development of highly specific biomarkers for particular stages of acute appendicitis facilitates clinical decisions and may be important in the diagnosis of acute appendicitis. Studies have also shown that people with acute perforated appendicitis experience an increase in IL-8 levels in their serum, demonstrating the effectiveness of IL-8 as a diagnostic marker for advanced appendicitis (Daneshmandi *et al.*, 2009; Marianiet *al.*, 2008). Some research have established a link between appendicitis and IL-8, whereas others have only

connected it to complex appendicitis. (Groselj-Grence *et al.*, 2007)

Immunohistochemistry is the use of monoclonal and polyclonal antibodies for the detection of particular antigens in tissue sections. Immunohistochemistry (IHC) is based on the principle of using monoclonal and polyclonal antibodies to determine the distribution of an antigen in tissues in health and disease. IHC is crucial in pathology, especially in the fields of hematopathology, neuropathology, and oncologic pathology (Dhamia *et al.*, 2017). Immunohistochemistry was used in this investigation to examine formalin-fixed, paraffin-embedded tissues to determine the expression of IL-8 in appendicitis. All of the fifty (n=50) appendix tissues stained positive for IL-8. The results of the study indicate that the proinflammatory chemokine IL-8 is strongly expressed immunohistochemically in appendicitis. According to the results of the immunohistochemical analysis, IL-8 positivity was demonstrated by the precipitation of brownish deposits in the cytoplasm. The study also found that there were no age, season, or gender differences in the immunohistochemical expression of IL-8 in appendicitis patients. In other words, IL-8 was produced by people of every age and gender. The results of this study are consistent with those of Kishimoto (2010), who claimed that an increase in IL-6 and IL-8 production played a role in the pathogenesis of several chronic inflammatory and autoimmune diseases. Kishimoto (2010) also claimed that IL-6 and IL-8 play a significant role in several human disease states, including cardiovascular diseases, sepsis, fever, cachexia, insulin resistance, osteoporosis, and neurological disorders.

Appendicitis is typically a disease of youth because it is more common within the age ranges of 10 and 30. The age distribution in both sexes follows a similar pattern, supporting the idea that neither sex nor the X chromosome predisposes people to appendicitis (Ayoade *et al.*, 2006; Noudeh *et al.*, 2007; Oguntola *et al.*, 2010). Its peak occurrence is between the ages of 10 and 14 for females and 15 to 19 for males (Papandria *et al.*, 2013). According to Duduyemi (2015), acute appendicitis affects more women than men but acute appendicitis with peritonitis affects more men than women. Mangete and Kombo (2004) discovered a considerably greater incidence of appendicitis among females in Port Harcourt, Nigeria. The findings of this study agree with the study by Mangete and Kombo (2004) and Duduyemi (2015) which observed that appendicitis is significantly more common in women than men. This study also demonstrates that appendicitis was more prevalent between the ages of 10 and 19 (35.4% of female patients, and 24.4% of male patients). This finding is completely consistent with that of Barlas *et al.* (2010), who found that appendicitis was more prevalent in individuals aged 10 to 19 and that it was less common in those 50 and above, while perforation was frequent in the 0–9 and 50–plus age groups.

In this study, the highest number of male survey subjects (n = 25) were found in the year 2019 whereas the highest number of female survey subjects (n = 36) were found in the year 2015, which also had the largest number of overall participants (58) in the study period of 2015 to 2020.

Similar to seasonal variations, appendicitis cases were seen in clinics throughout the year, however certain months

are linked to greater rates, albeit this varies by area (Al Omran *et al.*, 2003). Many studies have found that the summer months are connected with higher appendicitis incidences (Gallerani *et al.*, 2006; Noudeh *et al.*, 2007). Sanda *et al.* (2008) have proposed intense challenge to the mucosa-associated lymphoid tissue from allergens in the dust, during the sandstorms of the spring months as a cause of appendicitis in the Arabian Peninsula, despite some studies reporting an increase in appendicitis during spring and implicating a high prevalence of viral infections among others during these months.

Oguntola *et al.* (2010) reported that cases of appendicitis attain highest peak between the months of April to September, and declined from the month of October, reached their lowest level in December, and then slowly increased from the month of January. This finding is not in agreement with the common findings of decreasing incidence in a larger portion of the developed world. In terms of perforation, Barlas *et al.* (2010) observed that perforated appendicitis was more common in the summer and fall than non-perforated appendicitis. Their research found that whereas perforation occurred less frequently in the winter, appendicitis occurred more frequently. The coldest three months of the year, December, January, and February do show a statistically significant relationship between temperature and appendicitis perforation.

In this study, the impact of seasonal variations on the incidence of appendicitis in both male and female respondents revealed a substantial decline in appendix cases among males in 2015 in summer and winter. Likewise, a significant drop was recorded in the number of appendectomies performed in females in summer and winter. In contrast, a statistically non-significant increase was observed in 2018 in both genders and in 2019 in mainly females, while non-significant decreases in both genders was recorded in 2017 and 2020. This study has a few drawbacks that should be acknowledged. First, only fifty formalin-fixed, paraffin-embedded tissue blocks were used for the immunohistochemical studies due to financial limitations. Another restriction might be the exclusive use of immunohistochemistry without gene expression analyses. Immunohistochemical analyses, on the other hand, evaluate the expression of the protein that results from the last stage of gene expression. Further research is required to examine the changes in gene expression between various forms of appendicitis and to comprehend the genetic susceptibility to acute appendicitis and its pathophysiology for a better understanding of gene expression in the full spectrum of appendicitis is necessary.

In conclusion the study establishes that IL -8 cytokine was expressed in appendicitis and the condition is more common among women than men with higher incidences in summer.

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