The effect of various forms of treatment of vasculitis on C_3, C_4 and C_{5a} complement levels in infants and children attending Assiut University Children Hospital (AUCH)

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Abstract: Vasculitis is an umbrella term for various and heterogeneous disorders sharing the presence of inflammation of blood vessel walls (Geetha & Jefferson, 2020). Immune cell infiltrates involve the presence of leukocytes in the vessel with immune-complex deposition, which implies the activation of the complement system and then the swelling and destruction of vessel mural structures. The lumen is narrowed or occluded leading to ischemia and necrosis (Chimenti et al., 2015). It produces local symptoms resulting in hypoperfusion, infarction and hemorrhage and systemic symptoms with an increase in acute phase reactants (Salvador, 2020).

According to the size of the blood vessel affected and the distribution of vascular injury, pediatric vasculitis is classified into small vessel vasculitis as in Henochonlein Purpura (HSP), medium-sized vessel vasculitis as in Kawasaki disease and large vessel vasculitides affecting the aorta and its proximal branches. Some forms of small vessel vasculitis are characterized by the presence of antineutrophil cytoplasmic antibodies (ANCAs), whereas others are associated with immune complex deposition in affected tissues. Clinical presentation supported by specific laboratory test, imaging, and confirmatory histology are necessary in order to perform vasculitides' diagnosis (Sivaraman et al., 2020). To assess the severity of vasculitis and response to treatment, urinary biomarkers such as albumin/creatinine ratio (A/C ratio) in urine have been proved to be easily done and correlated well with both clinical and serological results (Zhang, 2020).

Aim of Work: The aim of this study is to assess the effect of various forms of drugs, used in treatment of vasculitis, on the serum levels of complements (C3, C4, and C5a) and Antineutrophil cytoplasmic antibodies (ANCA), in infants and children attending Assiut University Children Hospital (AUCH).

Conclusion: In conclusion, cases on combined Methotrexate and Steroid therapy scored best regarding the lowering of C5a level in serum, however, in our cases as a whole, the level of C5a didn't differ significantly from its level in control.

Although the 3 arms of therapy used in this study had good effect on C3 & C4 levels, yet their effect on C5a level was not significant from control. This finding could be explained by the fact that most of our studied cases were of the immune-complex deposition diseases type (which have no effect on C5a)

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and were ANCA negative. Therefore, C5a inhibitor drugs probably will not be suitable in treatment of most common causes of pediatric vasculitis encountered in this series. Perhaps, this type of therapy might be used in ANCA-Positive associated vasculitides, e.g. Wegener's Granulomatosis. Further research with bigger number of ANCA positive cases is needed to decide whether such cases could benefit from the use of C5a inhibitors or not.

Keywords: Vasculitis, ANCA, C5a, complement, Albumin/ creatinine Ratio, AAV, Corticosteroids, Cyclophosphamide, Methotrexate.

1. Introduction

Ford & Monach, 2019, defined the vasculitides as inflammatory affection of arterial blood vessels by polyangitis. The pathogenesis of vasculitides is related to the presence of leukocytes in the vessels and to the immune complex deposition. Following immune complex deposition, complement system (especially C3a, C5a) is activated, triggering the recruitment of inflammatory cells including neutrophils. Adhesion molecules are expressed within vasculitic lesions with activation of lymphocytes and macrophages. This triggers further complement activation and cytokine production, which serve to induce further adhesion molecules, thus perpetuating the vascular destruction. Swelling and damage of vessel mural structures occurs, with narrowing or occlusion of vessel lumen, leading to ischemia and necrosis (Ting, 2014).

On the other hand, Antineutrophil cytoplasmic antibodies (ANCA) are immunoglobulin G (IgG) autoantibodies directed against constituents of neutrophil granules leading to neutrophil degeneration which results in cell apoptosis known as "Natoptosis" (NaTosis) of the cells. These lead to vessel endothelial cell damage. So that, ANCA formation seems to be the basic reaction in vasculitis (Gou etal, 2012 and Kallenberg and Heeringa, 2018). Kallenberg and Heeringa, 2018, suggested that complement activation at C3 and C4 was involved in renal damage ANCA associated vasculitis (AAV).

The trend in classification of vasculitis into: small-sized vessel, medium-sized vessel, and large sized vessel vasculitis, as well as into ANCA-associated vasculitis and ANCA-negative vasculitis have been highlighted by Rhee & Merkel, 2020. Small vessel vasculitis (SVV) refers to intraparenchymal arteries, arterioles, capillaries, venules and veins; hence is associated with edema in some of them as in HSP and post-infectious vasculitis (Ford & Monach, 2019). SVV can be further subclassified into two major divisions depending on the pathogenesis: The antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) with few or no immune deposits, e.g. Granulomatosis with polyangitis (Wegener granulomatosis) (GPA (WG)) and the immune complex (IC) mediated small vessel vasculitis (SVV). The later includes HSP with IgA immune complex. While in SLE, the immune complex is classified as IgG mediated and hypocomplementemic immune complex disease. Other diseases with small vessel vasculitis include autoimmune diseases with specific human leukocyte antigen HLA affection such as SJIA (Sivaraman et al., 2020).

Vasculitis, also, can be a symptom of other underlying disorders triggering the inflammatory process (secondary vasculitis: as with infections, medications, toxins/allergens, malignancies, autoimmune conditions and connective tissue diseases etc.) or it could be the underlying cause of organ specific or systemic disease. In the latter case, the term "primary vasculitis" is used (Schnabel & Hedrich, 2019).

Not only the therapy in vasculitides is related to the size of the vessel affected, but also, it depends on the type of immune reaction produced and the type of immunoglobulin involved in the pathologic process; whether IgA, IgM, IgG, or others e.g. IgE. Furthermore, therapy

must take in consideration whether the complement system and/or the T-cells, neutrophils and lymphocytes are involved in the process of angiitis. Determination of the severity of renal and/or other organ granulomatous formation must be done (Frank and Hester, 2014). The authors also considered the formation of immune complexes has some bearing on the choice of therapy whether complement inhibitors as C5a inhibitors (e.g. Infliximab), or immunosuppressive agents. Zhang, 2020, suggested that assessment of the effect of therapy on vasculitis may be done by measuring the albumin/creatinine ratio (ACR) in urine and stated that it is correlated well with both clinical and serological results.

2. Patients and Methods

1. Type of Study Design:

The study is prospective hospital-based study, comparative randomized interventional clinical trial, its primary purpose was treatment (study phase 3). **ClinicalTrials.gov ID: NCT03692416**.

2. Study Setting & Duration:

The study was carried out on children with manifestations of vasculitis attending Assiut University Children Hospital (AUCH) over a period of 2 year (from Nov. 2019 to Nov. 2021).

3. Study Subjects:

Demographic data of participants & Recruitment:

The study was carried on 70 participants diagnosed with vasculitis attended Assiut University Children Hospital; 20 of them were studied initially before the onset of therapy (group I) and 50 children with pediatric vasculitis were studied while on treatment to see the effect of the drugs used in treatment (group II). Study also included 20 apparently healthy children, of matched age and sex to the patients, studied as control (group III). The children on study were 40 males and 50 females. Their age ranged from 2 to 16 years.

Inclusion criteria:

Infants and children diagnosed with vasculitis attended AUCH aged > 1 mo. and ≤ 17yr. of both genders were included during 2 years of study. They were diagnosed as vasculitis according to this paper (Schnabel A, Hedrich CM. Childhood Vasculitis. Front Pediatr. 2019 Jan 10;6:421. doi: 10.3389/fped.2018.00421. PMID: 30687686; PMCID: PMC6335362).

Exclusion criteria:

Those cases aged less than one month are excluded from the study.

4. Sample Size Calculation:

Sample size results obtained from Open Epi, Version 2, open source calculator—SSPropor, depending on the prevalence of vasculitis in Upper Egypt according to this paper (Shahin, et al. The distribution and outcome of vasculitic syndromes among Egyptians: A multi-center study including 630 patients. *The Egyptian Rheumatologist* (2018); 1-6).

$$\text{Sample Size } n = [\text{DEFF} * Np(1-p)] / [(d^2 / Z^2_{1-\alpha/2} * (N-1) + p*(1-p)]$$

Study Measures:

- **Groups:**

• The study included 70 participants diagnosed with vasculitis; 20 of them were studied initially before the onset of therapy (group I) and 50 children were studied while on treatment (group II). Study also included 20 apparently healthy children, of matched age and

sex to the patients, studied as control (group III). Group II participants (on treatment group) were subdivided into 3 arms according to the drug given to them: The 1st arm of patients received Glucocorticoids only, the 2nd arm received Glucocorticoids plus Methotrexate and the 3rd arm received Glucocorticoids plus Cyclophosphamide.

- **Arms:**

- **1st Arm (Glucocorticoids only):** Patients in this group received Prednisone Oral or Methylprednisolone IV:

1. Prednisone (for mild/moderate cases):
• Oral, on full stomach.
• Single daily morning dosage of 0.5-2.0 mg/kg/day or in 2-4 divided doses, max 80 mg/d.
• Duration: 4-6 weeks, with gradual tapering to the lowest effective dose.
2. Methylprednisolone (for severe/acute cases):
• IV injection
• 10-30 mg/kg/dose (max 1 g), over 1 hr daily for 1-5 days, followed by oral prednisone, 1-2 mg/kg/day, in 3 divided doses, with gradual tapering to the lowest effective dose. The duration is variable according to the condition of the patient. We adjusted the initial dose until a satisfactory response is obtained; then, we gradually decreased the dose in small decrements at appropriate intervals to the lowest dose that maintains an adequate clinical response.

- **2nd Arm (Methotrexate Plus Glucocorticoids):** Patients in this group received combined Methotrexate (SC) and Glucocorticoids (oral):

- Methotrexate:
• By subcutaneous injection.
• At a dosage of 10 to 20 mg/m2/wk (0.35 to 0.65 mg/kg/wk), max dose 25 mg/wk, Plus:
- Glucocorticoids:
• Oral Prednisone: 1-2 mg/kg/day, in 3 divided doses, with gradual tapering of dose over the last 2-3 weeks.
• Duration of therapy: 6-12 wks of combined Methotrexate plus Glucocorticoids therapy, with gradual tapering of steroids over the last 2-4 wks.

- **3rd Arm (Cyclophosphamide Plus Glucocorticoids):** Patients in this group received combined Cyclophosphamide (IV) and Glucocorticoids (oral):

- Cyclophosphamide:
• By Intravenous (IV) injection.
• At a dosage of 500-750 mg/m2/dose, monthly; not to exceed 1 g/m², Plus:
- Glucocorticoids:
• Oral Prednisone: 0.25-1 mg/kg/day, once daily, with gradual tapering of dose over the last 4-6 weeks.
• Duration of therapy: 8-12 weeks of combined Cyclophosphamide plus Glucocorticoids therapy, with gradual tapering of steroids over the last 4-6 weeks.

- **Assigned intervention:**

Besides full clinical history and detailed accurate clinical examination, all cases and control had the following investigations done: Complete blood counting (CBC), C-reactive protein (CRP), and Erythrocyte sedimentation rate (ESR), as well as serum levels of C3, C4, and C5a complements. Also, blood levels of antinuclear antibodies (ANA), anti-double stranded DNA antibodies (Anti-ds DNA) and antineutrophil cytoplasmic antibodies (ANCA) were measured. Serum urea, creatinine and urinary albumin/creatinine ratio were also done.

- **Study Materials:**

Laboratory Procedure

Seven milliliters (ml) of venous blood were collected under complete aseptic conditions then:

- Two ml of blood were added into EDTA coated tube, for complete blood count and sedimentation rate.
- Five ml of blood were added into plain tube, left to clot for 30 min at 37^0 C and centrifuged at 3000 rpm, the separated serum was used for other routine investigations and C5a determination.

Routine investigations

a. Complete blood counting (CBC) using automated blood cell counter (Celltac α, Japan)
b. Erythrocyte sedimentation rate (ESR) by Westergren method.
c. Serum urea, creatinine, albumin and urinary creatinine were performed on Beckman Coulter AU480 chemistry analyzer, USA. Reagents supplied by BM Egypt Company.
d. C-reactive protein (CRP) in mg/L, C3 and C4 in g/L were done by nephylometric method on BN Prospec system, Siemens Healthineers Global.
e. Antinuclear antibodies test (ANA) was done by ANAFLUOR™ indirect fluorescent antibody test, Diasorin, USA.
f. Anti-double stranded DNA antibodies test (Anti-ds DNA) was done by nDNAFluoro-Kit™, indirect fluorescent antibody test, Diasorin, USA.
g. Antineutrophil cytoplasmic antibodies (ANCA) screen index was done by automated ELISA on ALEGRIA Orgentec, Germany.

3. Specific investigations

Human complement 5a

Quantitative determination of C5a concentration was done by SinoGeneClon Biotech Co.,LtdEnzyme-Linked Immunosorbent Assay (ELISA)Kit, China. Catalog no. SG-11614.

Principle

Solid phase sandwich ELISA. It utilized a monoclonal antibody specific for Human Complement C5a coated on a 96-well plate. Standards and samples were added to the wells, and any Human Complement C5a present bound to the immobilized antibody. The wells were washed and a horseradish peroxidase conjugated rabbit anti-Human Complement C5a polyclonal antibody was then added, and produced an antibody-antigen-antibody "sandwich". The wells were again washed and TMB substrate solution was loaded, which produced color in proportion to the amount of Human Complement C5a present in the sample. To end the enzyme reaction, the stop solution was added and absorbances of the microwell were read at 450 nm. The concentration of C5a is then determined by comparing the O.D. of the samples to the standard curve.

Calculation of result

Concentration of sample (pg/ml) X 5 (dilution factor)/1000= sample concentration (ng/ml)
Control's results ranged from 0.22 ng/ml to 5.88 ng/ml
Patient's results ranged from 0.06 ng/ml to 9.54 ng/ml

Statistical Analysis of Data

- **Data collection:**

All data was collected by the researcher in a clinical sheet from the parents of the patients with symptoms and signs of vasculitis attending Assiut University Children Hospital over 2 years of study.

- **Data management and statistical analysis:**

Computer software was performed using SPSS (windows statistical package for the social science, version 23, IPM, and Armonk, new work). Quantitative variables were presented in terms of median or mean ±2 stander deviation. Qualitative variables were expressed as frequency and percentage. The Cutoff point of P values less or equal 0.05 were considered of statistical significance.

Ethical Considerations:
All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee. The study was approved by the corresponding Ethical Review Board at Faculty of Medicine, Assiut University **(IRB no: 17200271)** and informed consent was taken from patient's parents. Patient privacy and data confidentiality was ensured in all steps of the study. We are grateful for Assiut University for guiding us in publishing the present study.

4. Results

The study was carried on 70 participants diagnosed with vasculitis; 20 of them were studied initially before the onset of therapy (group I) and 50 children with pediatric vasculitis were studied while on treatment to see the effect of the drugs used in treatment (group II). Study also included 20 apparently healthy children, of matched age and sex to the patients, studied as control (group III). The children on study were 40 males and 50 females. Their age ranged from 2 to 16 years. Group II participants (on treatment group) were subdivided into 3 arms according to the drug given to them: The 1st arm of patients received Glucocorticoids only, the 2nd arm received Glucocorticoids plus Methotrexate and the 3rd arm received Glucocorticoids plus Cyclophosphamide. It was observed that Glucocorticoids alone were used in 58% of cases; Glucocorticoids plus Methotrexate were used in 26% of cases and Glucocorticoids plus Cyclophosphamide were used in 16% of the cases.
In the present study, SLE was the most frequently encountered cause of vasculitis, representing 27.1% of the cases studied. While, SJIA represents 25.7% of the cases, HSP represents 22.9% of the cases, and post-infectious vasculitis was found in 12.9% of the cases studied. Other causes of vasculitis encountered in this study are Dermatomyositis, Wegener's Granulomatosis, gangrenous vasculitis, and lastly, vasculitis in Raynaud's disease.

Table 1: Comparison between cases of vasculitis: initial cases (group I), those on treatment (group II) versus control (group III) regarding ANCA titer, Albumin/Creatinine Ratio (ACR), serum C_3, C_4 & C_{5a} levels

Median (range)	Groups			p-value*		
	I- Initial (n= 20)	II- Cases on treatment (n=50)	III- Controls (n= 20)	(I vs. II)	(I vs. III)	(II vs. III)
ANCA titer	12.00 (6.00-160.00)	10.00 (10.00-320.00)	7.00 (3.00-10.00)	0.427	**<0.001***	**<0.001***
Albumin/Creatinine Ratio (ACR) (mg/g)	65.65 (13.77-16934.00)	54.35 (10.80-20198.60)	15.63 (7.30-48.10)	0.594	**<0.001***	**<0.001***
Serum C3 level (mg/dl)	110.35 (10.20-190.00)	105.07 (3.20-215.00)	135.00 (31.70-200.30)	0.995	0.099	0.083
Serum C4 level (mg/dl)	10.55 (1.00-55.40)	18.25 (1.12-47.30)	26.60 (2.90-45.00)	0.405	0.068	0.239
Serum C5a level (ng/ml)	0.42 (0.06-5.29)	0.47 (0.17-9.54)	0.67 (0.19-5.88)	0.342	0.107	0.224

Data expressed as median (range)
*Comparison significant between each two groups by Mann Whitney test

It was observed that both initially studied patients and those on treatment showed significantly higher levels of ANCA and A/C ratio than in control with P value less than 0.001 in both, while no significant difference between patients studied initially and those on treatment regarding these variables.

Table 2: Comparison between different types of therapy used in cases with vasculitis:

Median (range)	Groups			p-value*		
	I- Steroids only (n=29)	II- Methotrexate + steroids (n=13)	III- Cyclophosphamide +Steroids (n=8)	(I vs. II)	(I vs. III)	(II vs. III)
ANCA titer	10.00 (10.00-160.00)	10.00 (10.00-80.00)	25.0 (10.00-320.00)	0.595	**0.024 ***	**0.033 ***
Albumin/Creatinine Ratio (ACR) (mg/g)	68.10 (16.00-20198.60)	27.50 (10.80-10322.10)	575.00 (11.30-16934.50)	**0.023 ***	0.376	0.082
Serum C3 level (mg/dl)	125.09 (10.40-176.10)	101.20 (3.20-215.0)	55.50 (20.30-149.00)	0.693	0.083	0.169
Serum C4 level (mg/dl)	18.30 (1.68-42.90)	21.40 (1.12-47.30)	7.750 (1.61-30.80)	0.957	0.113	0.277
Serum C5a level (ng/ml)	0.52 (0.17-9.54)	0.46 (0.21-3.03)	0.50 (0.36-6.35)	0.369	0.941	0.365

Data expressed as median (range)
*Comparison significant between each two groups by Mann Whitney test

Regarding ANCA titer, it was observed that cases on steroids only (I) and cases on steroids + Methotrexate (II) scored significantly better than cases on steroids + Cyclophosphamide (III), with P value less than 0.024 and less than 0.033 respectively.
Regarding Albumin/Creatinine ratio (ACR), it was also observed that cases on steroids + Methotrexate (II) scored significantly better than cases on steroids only (I) with P value less than 0.023.

Figures:

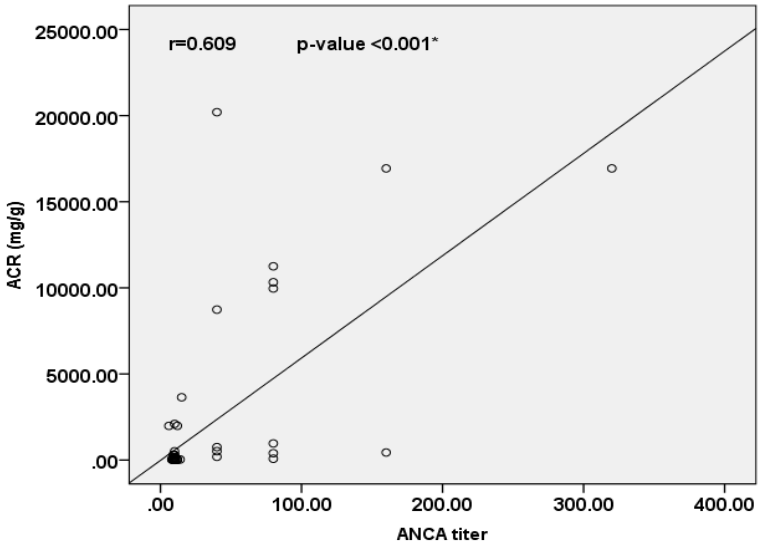

Figure (1): Regression curve showing significant positive correlation between ACR and ANCA titer in cases of vasculitis with P value < 0.001.

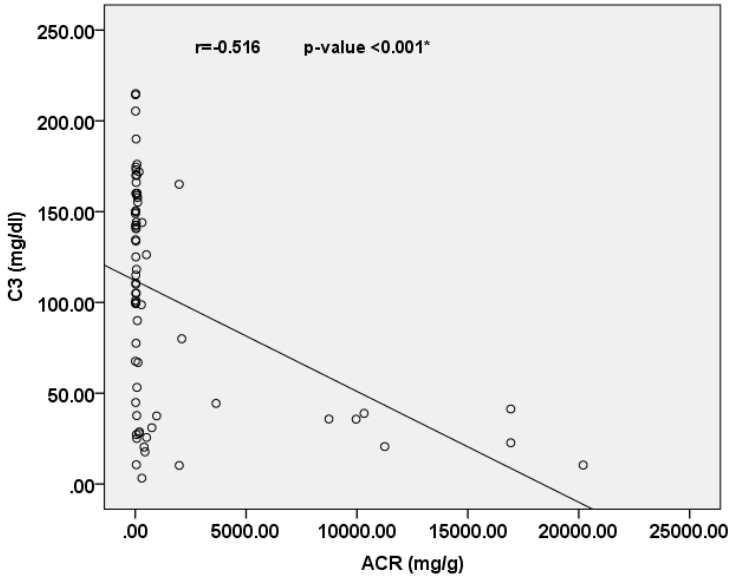

Figure (2) Regression curve showing significant negative correlation between ACR and C3 in cases of vasculitis with P value < 0.001.

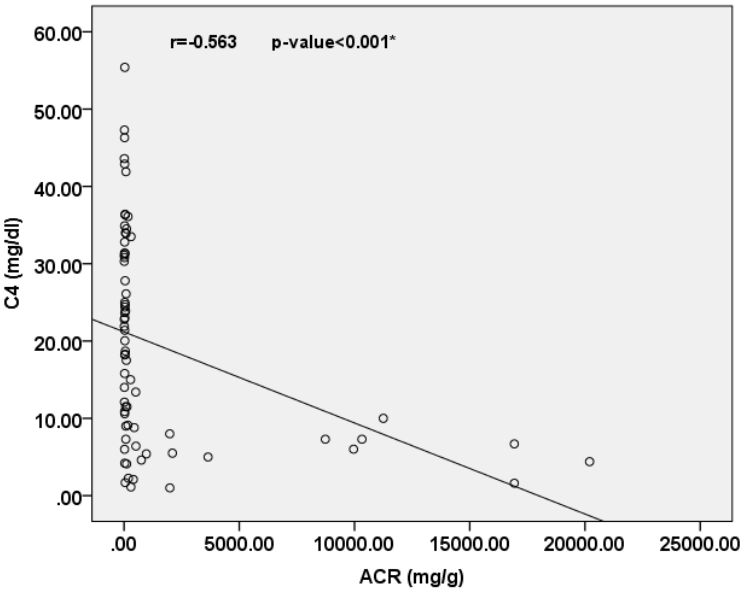

Figure (3) Regression curve showing significant negative correlation between ACR and C4 in cases of vasculitis (initial cases and those on treatment), with P value < 0.001.

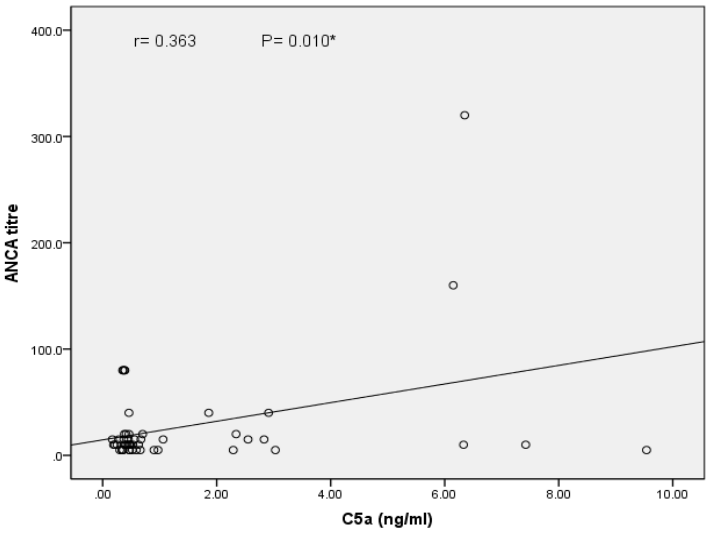

Figure (4): Regression curve showing significant positive correlation between ANCA titer and C_{5a} level (ng/ml) in patients on treatment group

Discussion

The diagnosis of vasculitis type is based on clinical and laboratory as well as on classification by the degree of renal affection as based on renal biopsy. Renal biopsy is a histopathologic invasive procedure and is considered a major drawback. Another classification is based on whether renal affection is in the form of immunocomplex mediated or complement mediated

disease. This classification can facilitate understanding which complement component is affected.

The causes of vasculitis in children – unlike those in adults – are more often primary than secondary as in malignancy, inflammatory bowel disease, and in cases of uveitis in the eyes e.g. in Kawasaki disease [American Academy of Pediatrics (ACP)]. The reaction between infection as in post-infectious vasculitis (reactive arthritis and vasculitis) has been recently reported; Bomback, 2014. The author reported that unintended infectious adverse events cause stimulation of the earlier components of complement system C3 and C4, and the potential need for lifelong therapy. Furthermore, this may be ticked over by being predisposed to such reaction by a special HLA typing as in systemic idiopathic juvenile arthritis (SJIA) (previously known as systemic rheumatoid arthritis or Still's disease).

In the present study, SLE, SJIA, HSP and post-infectious reactive vasculitis constituted more than 80% of the causes of pediatric vasculitides. Post-infectious vasculitis has been described after bacterial, viral, fungal or even after parasitic infections including amebiasis and schistosomiasis. In all these cases, lymphocyte abnormality or abnormality in the immune system is suspected (Kliegman, 2020); whether the complement system is involved in this type of pediatric vasculitis is still unknown.

Common infections that may be followed by vasculitis are salmonellosis, meningococcal, fungal or viral infections such as hepatitis C or more recently Covid 19 virus (Becker, 2020), COVID-19 is a SARS–CoV-2 syndrome that can involve all organs, including the circulatory system. Endothelial cell inflammation occurs within arteries, arterioles, capillaries, venules and veins and contributes to pathological events; including tissue hypoperfusion, injury, thrombosis and vascular dysfunction in the acute, subacute and possibly chronic stages of disease. Kawasaki-like disease with accompanying toxic shock syndrome or multi-systemic infammatory disease has been reported in children with COVID-19 (Becker, 2020). A pattern of tissue damage consistent with complement-mediated microvascular injury and thrombosis was noted in the lung and/or skin of 5 individuals with severe COVID-19 (Margo, et al., 2020).

In table 1, It was observed that both initially studied patients and those on treatment showed significantly higher levels of ANCA and Albumin/Creatinine ratio than in control, with P values less than 0.001 in both, which indicates the importance of these tests in diagnosis of cases of vasculitis. In this respect, our results are in keeping with EULAR/PRINO/PRES classification criteria for pediatric vasculitides which documented the important role of ANCA & Albumin/Creatinine testing in diagnosis of these cases (Ozen et al, 2010 & Schnabel & Hedrich, 2019). Geetha & Jefferson, 2020 & Aringer, 2020, described the importance of these tests in diagnosis of vasculitis. On the other hand, there was no significant difference between patients studied initially and those on treatment regarding these variables; which can be explained by the short duration of therapy in patients studied and small patient number. Moreover, the same table showed that we did not find any significant difference between the 3 groups studied regarding serum levels of complements. Recently, Hakroush S, et al., 2021 showed that circulating levels of C3 and C4 in ANCA associated vasculitis were comparable to the majority of other renal pathologies. Furthermore, hypocomplementemia was only detectable in a minor subset of ANCA associated vasculitis and correlated with acute kidney injury (AKI) severity in these vasculitides, independent of systemic disease activity or extrarenal manifestation, in line with previous reports (Kallenberg and Heeringa, 2018). This point to the fact that, in spite of the improved understanding of complement disregulation in the pathogenesis of subgroups of glomerular diseases, yet, we have not seen fully matched effect by advance in treatment of these diseases up to date. It is worthwhile to mention that regarding C5a level studied in this series, no significant difference was found between cases and control; a finding that could be explained by the fact that most of our studied cases were of the immune-complex deposition

diseases as SLE, SJIA, HSP, and post-infectious vasculitides; which have minimal effect on C5a level. Further research on this point is needed to decide whether such cases could benefit from the use of C5a inhibitors or not. Although, Eculizamab and similar complement inhibitors, while they have revolutionized the treatment of diseases related to ANCA-associated +ve vasculitis, yet, it will not probably be an equally game-changing drug for the more common diseases of pediatric vasculitides, with immune complex glomerulonephritis.
In the present study, the relationship between compared drugs used in treatment of vasculitis is only a preliminary study and a future study with bigger number of cases are recommended with confirmation of initial and follow up skin biopsies to confirm our findings (see table 2).
Regarding ANCA titer, it was observed that cases on steroids only (I) and cases on steroids + Methotrexate (II) scored significantly better than cases on steroids + Cyclophosphamide (III), with P value less than 0.024 and less than 0.033 respectively. Regarding Albumin/Creatinine ratio (ACR), it was also observed that cases on steroids + Methotrexate (II) scored significantly better than cases on steroids only (I) with P value less than 0.023. These results agreed with other studies.
In the present study, the A/C ratio carried a positive correlation with ANCA level. So that, the higher this ratio (pointing to severer renal affection), the higher the ANCA titer in the blood as in granulomatous disease where response to C5a inhibitor drugs is likely to be present. This was made more obvious by the finding in the present series of a significant positive correlation between ANCA titer and C5a level (see figure 4).
In the present study, A/C ratio carried a significant positive correlation to ANCA titer levels and a significant negative correlation with C3 and C4 levels (see figures 1,2,3), with P value < 0.001 for each; in all patients; whether initially studied or those on treatment.
The study was limited by the diffuse study of wide variety of causes of vasculitides with different ttt modalities used, and future studies are needed to be specific. Also, short duration of therapy & small sample size were another limitation. No many studies made in this issue on pediatric patients. Further research on C5a levels in different cases of pediatric vasculitis is needed to assess different therapeutic modalities (as the pathogenesis and causes of vasculitis is different from adults).

Recommendations

1. A/C ratio and skin biopsy should be done in all cases with vasculitis, both initially and follow up of the response to treatment; for the diagnosis of the presence and severity of renal affection. We hope that skin biopsy will replace the more invasive renal biopsy both for diagnosis and treatment.
2. Cases on treatment with steroids only (I) and those on treatment with steroids plus Methotrexate (II) scored better than cases on steroids plus Cyclophosphamide (group III), (I versus III, P value < 0.024) and (II versus III, P value < 0.033).
3. Because this is only a preliminary study, future studies with bigger number of cases are recommended, to confirm the finding in the present series.

Conclusion

1- In conclusion, cases on combined Methotrexate and Steroid therapy scored best regarding the lowering of C5a level in serum, however, in our cases as a whole, the level of C5a didn't differ significantly from its level in control.
2- Although the 3 arms of therapy used in this study had good effect on C3 & C4 levels, yet their effect on C5a level was not significant from control. This finding could be explained by the fact that most of our studied cases were of the immune-complex

deposition diseases type (which have no effect on C5a) and were ANCA negative. Therefore, C5a inhibitor drugs probably will not be suitable in treatment of most common causes of pediatric vasculitis encountered in this series. Perhaps, this type of therapy might be used in ANCA-Positive associated vasculitides, e.g. Wegener's Granulomatosis.

3- Further research with bigger number of ANCA positive cases is needed to decide whether such cases could benefit from the use of C5a inhibitors or not.

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