Hematological Study of Infants Amoebiasis in Duhok City

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Abstract:
Out of 180 children, 60 (33.3%) have Amoebiasis infection as diagnosed by direct wet smear and Saturated Salt Solution (SSS). SSS method is more significant (P=0.001) in diagnosis of the disease. Number of children infected with Amoebiasis infection is higher in infants aged 1-6 months, but without any significant difference to ages 6-12 or 12-18 months. In contrast, infants aged 18-24 months are significantly different (P=0.01) as the infection rate is 16.6%. Gender also is seen to be reduced in significance (P= 0.001) for females aged 18-24 months. Blood profile of the involved infants has shown a significant variation (P=0.01) for all blood profile parameters (RBC (P=0.05), WBC (P=0.001), Lymphocytes (P=0.05), Granulated WBC (P=0.05), Hb (P=0.01) and Platelets counts (P=0.001). Many medicinal regimes are dependent in the treatment of Amoebiasis, Metronidazole (Flagyl) in significant variation (P=0.01), combination of Metronidazole and Bactrim.

Key words: Amoebiasis, Entamoeba histolytica, Infant, Children, Direct Smear, Saturated Salt Solution, Blood Profile.

Introduction:
Amoebiasis is considered as a worldwide distributed diarrheic disease especially among children. Infection commences soon after ingestion by sensitive patients the infective stage of the causative agent which is the cystic form of Entamoeba histolytica protozoan parasite via polluted water, food and various other possible mechanical vectors [1, 2, 3]. Entamoeba histolytica is the most known agent causing Amoebiasis among their genus. The parasite colonizes in the mucosal layer Gastro Intestinal Tract (GIT) with preferability too, leading to lysis, corrosion and bleeding [4]. Sometimes the parasite distributes to other organs such as liver and brain, causing a serious consequences with bad sequels [5,6].

Other parts of the disease is related with the zoonotic importance as it can be transmitted from human to animal and vice versa [7,8].

Pet dogs, rodents and domestic flies are considered as biomechanical vectors as well as reservoir hosts [9]. Cystic form of the parasite is the resistant stage to radical environment which plays an...
important role in epidemiology of the disease [10].

The disease isn’t studied in infant children below 2 years old in Duhok city of Kurdistan region. The following study is designed to be concerned with the distribution of the disease among infants, blood profile, evaluation of technical methods and the medical approaches in treatment of the disease.

Materials and Methods:
Samples Collection:
A-Fecal samples (n=180 from March-July) are collected from diarrheic infants aged less than 2 years old randomly, sex and addresses are considered, too. Parasitological analysis is performed within half an hour following samples collection by two assessment diagnostic techniques, namely direct wet smear and Saturated Salt Solution (SSS). Trophozoite and cystic forms are investigated [11].
B- Blood samples (n=60) are collected with aseptic condition. Venous blood in about 3-5 ml is drawn from each reliable infant. Heparinized tubes are used, Coulter method is dependent [12], RBCs (Red Blood Corpuscles), WBCs (White Blood Cells), Lymphocytes, Granulated WBC (GRA-WBC), Hb (Hemoglobin) and Platelets counts are included.
c-Medical management approaches are categorized according to authorized physicians.

Statistical Analysis:
OpenEpi V.2.3 statistical program is dependent for descriptive statistics analysis and ANOVA application for the obtained results.

Results and Discussion:
Out of 180 cases, only 60 are considered as Ameobiasis among various diarrheic children. The infection rate reaches 33.3% at all (Tab. 1). This rate of infection is adapted with previous studies [13, 14]. In neighboring countries the results (24.6-33%) are near to the current one [15, 16]. Other results in other countries differ (10-70%) as they are concerned with surveys or broad range of ages or other aims [17, 18, 19].

Generally, the chronological approaches in combination with the current research results is clarified that there are no improvement recorded in control and eradication of Amoebiasis. This means that either there is a decrease in the scientific knowledge or negligence of this disease compared to other diseases.

Table (1): Infants Ameobiasis Diagnosis In Different Methods.

| Number of cases | Results categories | Test       | Positive | Negative |
|-----------------|--------------------|------------|----------|----------|
| 180             | Ameobiotic cases N=60 (33.3%) | Direct Smear (DST) | 20       | 40       |
|                 | Saturated Salt Solution (SSS) | 60***      | 0        |
|                 | Non Ameobiotic cases N=120 (66.6%) | By Both above tests | 0        | 120      |

***P=0.001

Table one shows that SSS techniques are more reliable (P=0.001) in comparison with direct wet smear preparation. The significance of this comparison is recorded in many researches and tests [1, 2, 3, 20]. It is worthy to provoke the use of SSS method especially in developing and unsophisticated places. Both of direct wet smear and SSS methods are valuable for the diagnosis of Trophozoic and cystic forms of the parasite, but there are significant differences (P=0.001) for trophozoites in direct wet smear as well as for significance (P=0.001) of cystic forms by SSS method.

The above mentioned results are familiar to previous (40%) studies [4,9,10]. These findings show very clearly that condition of examination
achievement is very crucial in relation to the accuracy of the test as well as for the specificity and sensitivity of the test. Direct fecal examination may be needed to be duplicated or triplicated in comparison with SSS method.

Table two shows that number of infants who got infection is higher in the age group 1-6 months but without any significant difference to the age group 6-12 or 12-18 months, in contrast to children of the age 18-

Table (2): Infants Ages Infected With Ameobiasis.

| Infant age (months) | Cases No. | % Female | % Male | % |
|---------------------|-----------|---------|--------|---|
| 1-6                 | 20        | 33.3*** | 12     | 20** | 8  | 13.3 |
| 6-12                | 15        | 25      | 7      | 11.6 | 8  | 13.3 |
| 12-18               | 15        | 25      | 7      | 11.6 | 8  | 13.3 |
| 18-24               | 10        | 16.6    | 2      | 3.3  | 8  | 13.3 |
| Total               | 60        | 100     | 28     | 46.5 | 32 | 53.2 |

24 months who showed a significance (P=0.01) of the infection rate of reduce from 33.3% to 16.6%.

These results reflect the similarity of situation of others [21] who found the same findings but disagree to other studies [13, 14, 16]. These controversies might be related to individual variations that could be related to epidemiogeographical factors that affect distribution of the disease.

The relationship of age to infection rate is very obvious as it occupies to below 6 months of age (33.3%) with special attention to occurrence within one month of age and that could explain the increase risk factors around infants in this age. This finding agrees with Mondala et al. [22] and disagrees with Ahmed [14].

Gender of children shows no significance difference for male infection rate at any age, but the situation differs from females where the infection rate is significant (P=0.001) to be reduced to 3.3% from 20%. The effects of gender upon infection rate of infants Ameobiasis is never pointed before, but it could be attributed to the society tradition where the males are very thusiastic in situation that makes them at high risk of exposure to Ameobiac infection. This indication is shown previously [7,13,14] in various rates (10-25%).

Blood parameters of children (Tab.3) shows a significant variation (P=0.001) for all blood parameters in comparison with normal values [Tab.3]. Values of RBC counts and Hb. levels are shown to be reduced (P=0.05 and P=0.01 respectively) in children suffering from Ameobiasis. This is expected as Entamoeba histolytica protozoan parasite has the ability to invade the epithelial tissue of GIT leading to minor bleeding that lately could be developed to clear obvious hemorrhage as it will be diagnosed by blood blotches in the feces or as observed free RBCs within microscopic fields.

Table(3): Infants Ameobic Blood Parameters.

| Blood Parameters | Positive Ameobiatric cases (n=60) | Negative Ameobiatric cases (n=100) | Normal |
|------------------|-----------------------------------|-----------------------------------|--------|
|                  | $\bar{y}$, SD                     | $\bar{y}$, SD                     | $\bar{y}$, SD |
| WBC (10^3/L)     | 9.27±1.33***                    | 8.31±0.9                      | 6.5±2.5 |
| Lymphocyte (10^3/L) | 3.45±0.9**                         | 3.22±1.1                      | 5±1.5  |
| GRA (Granulocytes) (10^3/L) | 4.97±0.98*                        | 3.1±1.6                        | 4.6±0.99 |
| RBC (10^12/L)    | 4.24±0.75*                        | 6.1±1.5                        | 4.5±1.3 |
| Hb (g/dL)        | 11.28±2.91**                      | 14.2±0.53                     | 13.5±2.5 |
| PLT (10^9/L)     | 168.2±50.6***                     | 289.5±30.5                    | 250±61  |

$P<0.05$ $P<0.01$ $P<0.001$

These findings are absolutely agree with many references that explain the pathogenicity of amoebiatic causative agent [4,13,22].

WBC (P=0.001), Lymphocytes (P=0.01) and Granulated WBC (P=0.05) are have a dramatic changes into rising or reducing in advancing of amoebiatic
infection, which stimulate the immune system in all to respond suddenly as acute reaction and that is very clear for lymphocytes regulatory role and GRA as they are the first immune cellular that intersects with the parasitic infection. This is adequate with many advanced studies all over the world [4,11]. Platelets counts (PLT) decrease significantly (P=0.001) and this is show to be a de novo hematological response which may be related to some of the parasite metabolite to make blood component available for parasite viability. Lately, Table 4 clarifies that many tactics are dependent in treatment of amoebiatic cases by the native physicians, mostly depending upon Metronidazole (Flagyl) in significant variation (P=0.001), Tinidazole (Fasigyn), combination of Metronidazole and Bactrim antibiotic. The above mentioned medications are recommended by many medical references [2, 6, 7, 11].

The major problem in medical treatment of Amoebiasis seems to be a lack of recommendation for necessity of re-checking of patients after finishing prescribed medicinal course. This dilemma needs more attention as it may be the only available method to judge the efficacy of medicines in developing countries, such as the current research place.

Table (4): Treatment Regimes Of Infants Amebiastic Infection .

| Treatment regimes | Medicates                | Number of applicants |
|-------------------|-------------------------|---------------------|
| Regime 1          | Metronidazole (Flagyl)  | 40**                |
| Regime 2          | Tinidazole (Fasigyn)    | 12                  |
| Regime 3          | Metronidazole + Bactrim | 8                   |
| Total             |                         | 60                  |

**P=0.01

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دراسة بعض التغيرات الدموية لداء المتحولات الاميبية في الأطفال الرضع لمدينة دهوك

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الخلاصة:

أظهرت نتائج فحص خروج 180 طفلًا أن 60 (33.3%) منهم مصابين بداء المتحولات النسيجية باستخدام المسحة المباشرة الرطبة والمحلول الملح الشبيه. اظهر المحلول الملح الشبيه فرقاً معنويًا عالياً (P=0.001) في تشخيصه للمرض. ان عدد الأطفال المصابين بداء المتحولات الاميبية كان عالياً في الاعمار 1-6 أشهر عن الاعمار 6-12 و12-18 شهراً ولكن بصورة غير معنوية. وكانت الصورة معكوسة بالاعمار 18-24 شهراً إذ كان الفرق معنويًا (P=0.01) اذا انخفضت نسبة الاصابة من 33.3% الى 16.6%. وانخفضت نسبة السوء الامنا معنويًا (P=0.001) بالاعمار 18-24 شهراً كذلك. أظهرت الصورة الدموية فروقاً معنويًا (P=0.01) لكامل القراءات (انخفض عدد الكريات الحمر معنويًا P=0.05) وارتفاع عدد الخلايا البيضاء الكلي معنويًا (P=0.01)، والخلايا المناعية انخفضت معنويًا (P=0.05) وارتفاع عدد الخلايا البيضاء الحبيبي معنويًا (P=0.05) اذ انخفضت نسبة خضاب الدم معنويًا (P=0.01) كذلك انخفض عدد الصفائح الدموية معنويًا (P=0.01). ولذا لوحظ استخدام العديد من أنظمة المعالجات الهرمونية من خلال الاطباء المحليين واكثرها اعتماد المترونيدازول (الفلاجيل) وبصورة معنوية (P=0.01) ثم التينيدازول (فيسيجين) وكذلك خليط المترونيدازول مع المضادات الحيوية البكتريمية.

الكلمات المفتاحية: داء المتحولات الاميبية، الأطفال، الرضع، الاطفال، المسحة الرطبة المباشرة، المحلول الملح الشبيه، الصورة الدموية.