Hepatic Amyloidosis Secondary to Schistosomal Infection: Therapeutic Effect of Colchicines Alone or in Combination with Praziquantel

Introduction:
Schistosomiasis is a parasitic disease caused by infection with helminthes Schistosoma species. The arrival of eggs in the liver during Schistosoma infection initiates a protective granulomatous response. However, as the infection progresses, this response results in chronic liver complications including amyloidosis and fibrosis (Andrade, 2004) which are central events in progressive liver disease. This leads to cirrhosis, and associated morbidity and mortality caused by decompensation. Amyloidosis is a heterogenous group of fibrillary protein deposition disorders involving the extracellular matrix of various organ systems. The liver is a major site of amyloid deposition. Hepatic involvement in amyloidosis can occur in both primary and secondary forms of the disease. Although liver is often involved, clinically apparent disease is rare (Yellapu, et al., 2010). Liver amyloidosis is the results of an imbalance between synthesis and degradation of extracellular matrix proteins of the liver. Colchicines, a naturally occurring alkaloid, inhibited collagen synthesis and fibroblast proliferation when added to culture of fibroblasts isolated from Schistosoma mansoni infected liver and may therefore be useful in modulating schistosomal hepatic fibrosis (Mansour et al. 1988). Montasser et al. (1994) also suggested that Colchicine has therapeutic effect against schistosomal liver fibrosis. When colchicines were given concomitantly with Praziquantel (PZQ), early and late of infection. Biochemical and histopathological investigations were measured to assess the effect of Col or/ and PZQ treatment. Significant reduction in hepatic Amyloid deposits and improvement in serum albumin and cholesterol were observed when combined treatments were given. This was nearly complete with early treatments and only partial when treatment was given late. We conclude that, colchicine is effective for the prevention of and cure hepatic amyloidosis secondary to schistosomal infection.

Materials and Methods:

Experimental Design:
Male hamsters were used in this study as Hamed et al., (2007) recommend to use male hamsters when use this experimental animal in schistosomal infection research. Seventy hamsters were infected with ±200 S. haematobium, they were divided into three groups G2: infected not treated (positive control, PC); G3: infected with early treatment at 9 week post infection; G4: infected with late treatment at 15 week post infection. Another uninfected group was considering as negative control, NC (G1). Group 3 and 4 each contain 30 hamsters, were subdivided into three subgroup each of 10 hamsters differ according to the type of treatment (G3a, 4a) were treated with Colchicine only COL Group (G3b, 4b) were treated with COL + PZQ. Group (G3c, 4c) were treated with PZQ only. Five hamsters from each group were sacrificed at 18 week of infection and the other 5 hamsters at 24 weeks.

Drug Dosage:
Drug dosage was given according Sobh et al., (1995). Both drugs are given orally, Colchicine in a dose of 60 µg/ kg daily until the time of sacrifice and PZQ as 100 mg/ kg once which was repeated weekly for 3 consecutive weeks.

Biochemical and Histopathological investigations:
At sacrifice, animals were anesthetized, liver and spleen tissues were obtained and examined histopathologically. Liver and spleen specimens were fixed in 10% neutral buffered formalin and processed as paraffin sections. These were stained with hematoxylin and eosin, Masson's trichrome, periodic acid- Schiff and Congo red stains. Sections were examined by a pathologist unaware of the animal group. At sacrifice, mesenteric veins were explored for adult worms. Weekly estimates of serum total protein, albumin and cholesterol were measured to all hamsters involved in this study, A/ G ratio were calculated.

Statistical Analysis:
Biochemical changes in different treated group were compared with NC and PC using Student's t test. Pathological changes were given numbers (0- 3) according to the degree of amyloid deposits. A nonparametric method was used to analyze these changes using SPSS package. P value of < 0.05 was considered as significant.

Results:
Complete parasite eradication was achieved in the groups treated with PZQ alone or in combination with COL (G3, b, c and G4a, c) as no adult worms were detected in the mesenteric circulation. In PC or G2 and COL treated group (G3a and G4a) the mean adult worms recovered was 10.98 ± 7.4; 9.5 ± 7.1 and 14.75 ± 8.34 adult worms, respectively.

Table 1 show the biochemical changes observed in hamsters
sacrificed 18 weeks after infection in NC, PC and hamsters treated 9 weeks, G3 and 15 weeks, G4 post infection. A significant improvement in serum albumin and cholesterol in COL, PZQ and COL + PZQ subgroups was observed when compared to PC (P2). These observations were the same when a treatment was given 9 or 15 weeks of infection. When compare the same biochemical parameters at different time of treatment (P3) a better significant improvement in serum albumin and cholesterol was observed in groups treated at 9 weeks more than groups treated at 15 weeks of infection.

Table 1: Prophylactic (9 wks) and Curative (15 wks) Effect of Colchicines and/or Praziquantel on Biochemical Parameters Measured at 18 weeks post infection Between Different Groups.

| Groups   | Alb  | TP   | A/G  | Chol |
|----------|------|------|------|------|
| G1       | M    | 2.75 | 6.18 | 0.801| 124  |
| NC       | S.D. | 0.1  | 0.25 | 0.03 | 20   |
| G2       | M    | 1.91 | 5.65 | 0.483| 209  |
| PC       | S.D. | 0.44 | 0.73 | 0.125| 78   |
| G3a      | S.D. | 0.26 | 0.69 | 0.135| 32   |
| COL9     | S.D. | 0.10 | 0.26 | 0.124| 30   |
| G3b      | S.D. | 0.34 | 0.26 | 0.144| 22   |
| COL+PZQ9| S.D. | 0.34 | 0.26 | 0.144| 22   |
| G4a      | S.D. | 0.38 | 0.59 | 0.101| 29   |
| COL15    | S.D. | 0.00008| 0.93 | 0.0002| 0.001|
| P1       | 0.4  | 0.38 | 0.62 | 0.2  |
| P2       | 0.1  | 0.4  | 0.67 | 0.45 |
| G4b      | S.D. | 0.23 | 0.24 | 0.112| 33   |
| COL+PZQ15|     | 0.00009| 0.39 | 0.001| 0.9  |
| P1       | 0.01 | 0.48 | 0.007| 0.01 |
| P2       | 0.066| 0.5  | 0.003| 0.32 |
| G4c      | S.D. | 0.29 | 0.37 | 0.124| 34   |
| P1       | 0.92 | 0.0004| 0.007| 0.02 |
| P2       | 0.001| 0.002| 0.003| 0.07 |
| P3       | 0.001| 0.001| 0.0003| 0.41|

Table 2 show the biochemical changes observed in hamsters sacrificed 24 weeks after infection in NC, PC and hamsters treated 9 weeks, G3 and 15 weeks, G4 post infection. A significant improvement in serum albumin and cholesterol in PZQ and COL + PZQ subgroups was observed when compared to PC (P2) while COL treated group was the same like PC group. These observations were noticed when a treatment was given 9 weeks of infection but not when treatment was given 15 weeks of infection. When compare the same biochemical parameters at different time of treatment (P3) a better significant improvement in serum albumin and cholesterol was observed in groups treated at 9 weeks more than groups treated at 15 weeks of infection only in COL + PZQ subgroup.

Table 2: Prophylactic (9 wks) and Curative (15 wks) Effect of Colchicines and/or Praziquantel on Biochemical Parameters Measured at 24 weeks post infection Between Different Groups.

| Groups   | Alb  | TP   | A/G  | Chol |
|----------|------|------|------|------|
| G1       | M    | 2.91 | 6.45 | 0.821| 127  |
| NC       | S.D. | 0.19 | 0.28 | 0.05 | 36   |
| G2       | M    | 1.66 | 6.28 | 0.46 | 187  |
| PC       | S.D. | 0.39 | 0.58 | 0.05 | 37   |
| G3a      | S.D. | 0.28 | 0.67 | 0.124| 30   |
| COL9     | S.D. | 0.52 | 0.73 | 0.133| 30   |
| G3b      | S.D. | 0.12 | 0.31 | 0.028| 25   |
| COL+PZQ9| S.D. | 0.19 | 0.29 | 0.088| 9    |
| G4a      | S.D. | 2.85 | 6.07 | 0.882| 118  |
| COL15    | S.D. | 0.11 | 0.8  | 0.053| 47   |
| P1       | 0.00006| 0.001| 0.002| 0.002|
| P2       | 0.33  | 0.75 | 0.33 | 0.01 |
| G4b      | S.D. | 2.12 | 5.57 | 0.615| 190  |
| COL+PZQ15|     | 0.57 | 0.6  | 0.179| 35   |
| P1       | 0.001 | 0.002| 0.01 | 0.01 |
| P2       | 0.19  | 0.48 | 0.16 | 0.9  |
| G4c      | S.D. | 2.88 | 6.31 | 0.845| 134  |
| P1       | 0.81  | 0.31 | 0.63 | 0.58 |
| P2       | 0.0003| 0.0006| 0.0003| 0.003|
| P3       | 0.1   | 0.1  | 0.31 | 0.2  |

Table 3 show the semi quantitative evaluation of hepatic amyloid deposits detected at the end point of infection in PC and hamsters treated 9 weeks, G3 and 15 weeks, G4 post infection. No amyloid deposits were seen in ant of the nor-
Table 3: Significant of Colchicines and/or Praziquantel treatment on hepatic Amyloid deposits detected at the end point of infection.

| Group       | Liver | Spleen |
|-------------|-------|--------|
| G2          | M     | 1.66   |
| PC          | S.D.  | 0.5    |
| G3a         | M     | 1.55   |
| COL9        | S.D.  | 0.5    |
| COL+PZQ9    | S.D.  | 0.005  |
| G3c         | M     | 1.25   |
| PZQ9        | S.D.  | 0.7    |
| G4a         | M     | 2      |
| COL15       | S.D.  | 0.01   |
| G4b         | M     | 1.37   |
| COL+P15     | S.D.  | 0.51   |
| G4c         | M     | 1      |
| PZQ15       | S.D.  | 0.94   |

Discussion:
The evaluation of Colchicine effect on hepatic amyloid deposits biochemical and histopathological study was the main aim of the present study. We compare this effect when the drug was given alone or in combination with anti-schistosomal drug. These treatments were tried early 9 weeks of infection, where no true amyloid deposits were detected in different tissues (preventive) and late 15 weeks of infection, where true amyloid deposits were detected in different tissues. Complete histological and biochemical regression was achieved when combined treatment was given early, yet this was partial when the drugs (COL or PZQ) were given alone. When combined treatment was given after establishment of amyloidosis an incomplete but significant reduction in disease markers (amyloid deposits and serum albumin) was achieved. When Colchicine or Praziquantel were given alone after establishment of amyloidosis, no histological was observed, yet there was significant partial reduction in serum albumin and cholesterol.

Amyloidosis is not a single disease, but a series of diseases in which there is extracellular deposition of a protein, often leading to prominent end organ dysfunction. The mechanisms of Colchicine action in the therapy of amyloidosis are still unclear. Colchicine was suggested to have a therapeutic effect against schistosomal liver fibrosis (Montasser et al., 1994; Si et al., 1995; Jiang et al., 1996). With other combined treatment, augmented reduction of liver fibrogenesis was achieved (Al-Harbi et al., 2012). Its effect was greatly improved when its treatment was combined to the anti-schistosomal drug Praziquantel (Sobh et al., 1995; Badawy et al., 1996 & 1999). These findings were concise with our findings.

We conclude that Colchicine has a significant effect on Schistosoma induced hepatic amyloidosis, especially when it given early of infection and in combination with anti-schistosomal drug Praziquantel.

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