ChronoBiology Letter

Summer 2014 — 18th Edition

Diagnos-Techs™

Clinical & Research Laboratory Quarterly Newsletter #18

Articles:

News:

Did you know?

It is estimated that humans produce up to 1.5 liters of saliva during the day and virtually none while sleeping.

New design!

We have changed the layout of our ChronoBiology Letter to provide you with a more vibrant and enjoyable read. Along with the additions of Dr. Dialogue and TechTalk we will be slowly adding a few new sections in the coming editions. We welcome any feedback or suggestions that you may have to help us in providing you with the best newsletter.

Thanks - DTI Team

Where to find us:

AARM Association for the Advancement of Restorative Medicine

October 9-12
Santa Fe, NM

A4M American Academy of Anti-Aging Medicine

December 11-13
Las Vegas, NV

Salivary Cortisol - A Biomarker for Depression

John J. White MD, CM

Disturbances in the circadian rhythm of cortisol long have been recognized to be a marker of stress upon the body. Measurement of disturbances in cortisol response throughout the day underlies the Adrenal Stress Index (ASI) testing offered by Diagnos-Techs™. Lesser known, perhaps, is the value of cortisol evaluation as an objective marker for major depressive disorder (MDD), which is a debilitating mental health problem affecting one in six people at some time in their lives.

Dysregulated cortisol rhythms and elevated morning and evening cortisol values have been reported consistently as risk factors for, or consequences of, major depressive disorder.[1-6] A recent, much heralded, publication from Cambridge University has reported that an elevated 8^{AM} cortisol can serve as a predictive biomarker for major depression in boys with early depressive symptoms.[7]

These researchers note that "lack of precision in identifying valid population-based subtypes at differential risk for MDD is a serious impairment in targeting available interventions toward the most susceptible individuals." They also note the "low validity of elevated depressive symptoms alone as a recruitment index for early interventions." Consequently, suitable early interventions previously have been difficult to target appropriately for adolescents.

Studying a large adolescent population over 1-3 years, the Cambridge researchers noted significant elevation in 8^{AM} salivary cortisol values along with self-reported high current depressive symptoms in a subset of adolescent boys in the population. Teenage boys so identified were 14 times more likely to develop MDD, compared to boys with low morning cortisol and low depressive symptoms. By contrast, teenage girls with high depressive symptoms were found to be four times more likely to develop MDD, regardless of cortisol levels. Both boys and girls in these higher risk groups showed signs of mnemonic vulnerability, i.e. impaired autobiographical memory recall.

They concluded that the combination of high current depressive symptoms and elevated morning cortisol is particularly hazardous for teenage boys. Elevated morning cortisol may be used as an objective biomarker, along with current depressive symptoms, to help identify adolescent boys at risk for MDD and to direct appropriate therapeutic intervention at an early age.

It is now recognized widely that "salivary cortisol measurement offers an excellent reflection of the plasma free cortisol concentration in normality and disease because it circumvents the changes in total cortisol due to corticosteroid-binding globulin alterations."[8] Noninvasive salivary collection was

(Continued on page 3)

Amoebiasis

Kamal Henein, MD

The term amoebiasis refers to an infection with the parasite *Entamoeba histolytica*, a 10-60µm long protozoan that moves via finger-like extensions called pseudo-pods. *E. histolytica* exists in cyst form (infective) and trophozoite form (invasive), as shown in Figure 1. Roughly 50 million symptomatic cases of amoebiasis occur each year, with up to 100,000 deaths occurring worldwide.[1,3] Yet, since estimates were made before the molecular identification of pathogenic *E. histolytica* and non-pathogenic strains of *Entamoeba*, the prevalence of *E. histolytica* infection could be misleading.[4] After malaria, *E. histolytica* is likely the world's second leading protozoan cause of death.[5]

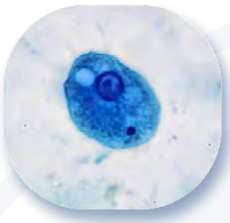


Fig. 1

The genus *Entamoeba* contains many species, and at least six of them *(E. histolytica, E. dispar, E. moshkovskii, E. polecki, E. coli, and E. hartmanni)* reside in the human intestinal lumen. *Entamoeba histolytica* is the only one of these species confirmed to cause disease in humans; the others are considered non-pathogenic as there is currently no evidence of a causal link between their recovery and gastrointestinal symptoms.[1, 5, 6]

Note that almost all these species can cause symptoms in patients with variable degrees of severity.

Entamoeba histolytica infections are commonly observed in tropical and subtropical regions of the world.[7,8] Risk factors for *E. histolytica* infection include overcrowding, low socioeconomic status, and poor sanitation levels. These factors likely contribute to a higher prevalence of amoebiasis in developing countries.

Most infections occur in Central and Western South America, Western and South Africa, and India.[9] In the U.S. and other developed countries, *E. histolytica* infections are rare and commonly seen in visitors returning from endemic areas, recent immigrants, homosexual men, and inmates of institutions.[10, 11, 12, 13]

Humans are the only reservoir for *Entamoeba histolytica* and the main route of transmission is fecal-oral, from cysts passing in the stool of patients with chronic infections or asymptomatic carriers. Analyses based on published data indicate that only ten to twenty percent of those infected with *E. histolytica* develop the invasive form of the disease (including dissemination beyond the gastrointestinal tract to remote organs), while the rest of cases remain asymptomatic but chronically pass cysts in their stools.[2, 3] These cysts can survive outside the body for weeks.

(Continued on page 4)

Salivary Cortisol: Continued from page 1

chosen as the only feasible collection methodology in this adolescent population study with repeated 8^{AM} sampling. Overall, salivary sampling is the best logistical way to carry out clinical cortisol testing over time and throughout the day.

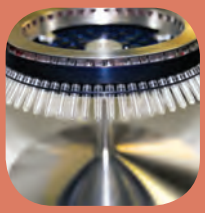
Insomnia is one of the major symptoms of MDD, and is often paired with anxiety. Unfortunately, this study did not consider possible cortisol aberrations at the end of the day. Frequently there is an evening (supper and bedtime) cortisol elevation in insomniacs, rather than the usual lower values typically seen with a regular circadian rhythm.

The Temporal Adrenal Profile (TAP) offered by Diagnos-Techs constitutes a suitable biomarker panel for adolescents with depressive symptoms, particularly boys, and other patients with sleep difficulties. Complete circadian cortisol sampling can provide more meaningful and complete information than an 8^{AM} sampling by itself.

*For all medical references please see back for details.

techtalk

Research & Development Updates



Advances in human genetics and molecular techniques are continually providing new insights into the links between genetics and human disease. For example, an increasing number of single nucleotide polymorphisms (SNPs), small differences in the human genome, have been linked to a broad spectrum of human disorders and diseases. Diagnos-Techs has made a significant investment in state-of-the-art molecular diagnostic equipment. Look to us in the near future to help you include clinically significant SNPs into the clinical picture of your patients, with cost-effective and noninvasive genetic testing.

Current Medical Topics

Dr. Dialogue

Testosterone:

Studies are showing an increase in the relative risk of myocardial infarction in the post-prescription interval (3 months), over the pre-prescription interval (one year) in men older than 65 years, and in younger men with heart disease. The Endocrine Society recommends that testosterone should only be prescribed for men with hypogonadism, and should be monitored to optimize the dose and control the risk of adverse effects.[1]

Older men with optimal (midrange) levels of testosterone and dihydrotestosterone have the lowest all-cause mortality rates.[2]

Vitamin D:

In children and adolescents, high total intake of vitamin D in the range of 240,000 to 4,500,000 IU can cause intoxication. Milder presentations of excess vitamin D intake have been reported in infants using currently recommended doses. Vitamin D supplementation at or above the upper range of the recommended dose, up to 400 to 1000 IU per day for children younger than one year of age and 600 to 1000 IU per day for children at least one year of age, should be monitored with 25-hydroxyvitamin D concentrations.[3]

A meta-analysis of 5 studies involving 4443 patients with breast cancer found that women with the highest levels of serum 25(OH) D at the time of diagnosis had almost double the survival rate as those with the lowest levels. Levels of vitamin D between 40 and 60 ng/ml are recommended for cancer prevention.[4]

Effect of Chronic Stress on Children:

Repeat exposure to violence, along with other types of physical and mental stress, has a cumulative effect causing an early erosion of telomeres. A cohort study of more than 200 British children found a linear correlation between frequency of exposure to violence and telomere length in children 5 to 10 years of age.[5,6]

(Continued from page 2)

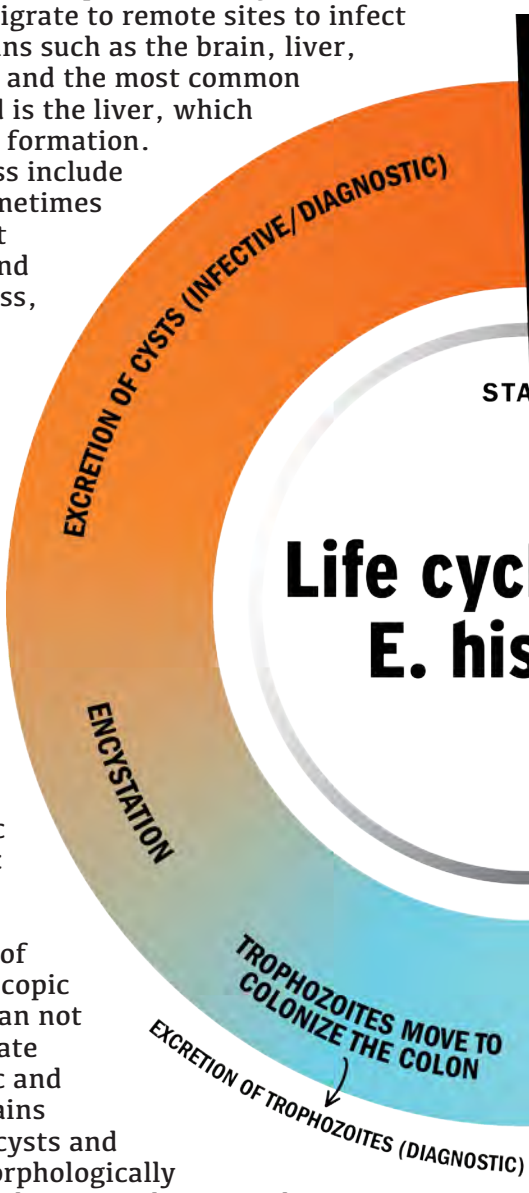
Three species of *Entamoeba* that colonize the intestine have similar cyst and trophozoite morphology: the pathogenic *E. histolytica* and the non-pathogenic *E. dispar* and *E. moshkovskii*.[6,8] Colonization with the non-pathogenic species occurs ten times more frequently than infection with the pathogenic species.[6] Amoebiasis occurs after swallowing the amoebic cyst (usually via contaminated food or water, although sometimes via direct person-to-person contact). Sexual transmission through oral-anal sexual practices have also been documented to cause infection, especially among same sex male partners. Contamination of food by infected food handlers and person-to-person contact appear to be the most significant means of transmission.

Within the colon, trophozoites can adhere to and destroy colonic epithelial and inflammatory cells, which may result in dysentery (characterized by blood and mucous in the stool). Symptoms of amoebiasis range from mild loose stools to severe dysentery;[14] however, the majority of infected individuals are asymptomatic. In rare cases, patients may present with intermittent diarrhea or constipation, abdominal pain, and flatulence; this presentation may be mistaken for inflammatory bowel disease (IBD).

To avoid misdiagnosis, patients with suspected ulcerative colitis or other IBD should be tested for *E. histolytica* before initiating therapy.

The pathogenicity of *Entamoeba* depends on multiple factors such as the specific strain, infective dose (a single cyst is enough to cause disease), and the host's genetic susceptibility including immune status and age.[15] Risk factors for severe disease include extremes of age, immunosuppressant medication, pregnancy, malignancy, and alcoholism. As demonstrated in Figure 2, following ingestion, the amoebic cysts, which are resistant to stomach acid, reach the terminal alkaline part of the small intestine. Here they excyst releasing trophozoites. The excystic form, or trophozoite, reproduces and migrates to the large intestine where it colonizes the lumen and feeds on bacteria, undigested food, and tissue debris. It is in this form that *Entamoeba* can disrupt the protective mucous layer overlying the colonic mucosa and invade the intestinal lining of the colon causing tissue damage, epithelial ulceration, bleeding, and colitis.

Research has identified a lectin protein on the surface of pathogenic *E. histolytica* trophozoites.[5, 16] This protein facilitates the adherence of trophozoites to the intestinal epithelium, invasion of the gut wall, and the spreading to extraintestinal sites. This protein also promotes mucosal immunity (IgA response) providing some protection to the intestinal lining. If penetration of the intestinal mucosal lining occurs, trophozoites may enter the blood stream and migrate to remote sites to infect extraintestinal organs such as the brain, liver, and lungs. The first and the most common organ to be affected is the liver, which may lead to abscess formation. Symptoms of abscess include abdominal pain (sometimes referred to the right shoulder), nausea and vomiting, weight loss, and intermittent fever and chills.



Evaluation of amoebiasis is typically done through microscopic stool examination, stool antigen detection, serology, salivary antibody testing, molecular testing, or colonoscopy. The differentiation between pathogenic and non-pathogenic amoebae is crucial for appropriate treatment. In spite of the fact that microscopic stool examination can not generally differentiate between pathogenic and non-pathogenic strains of amoebae[17] since cysts and trophozoites are morphologically identical for *E. histolytica*, *E. dispar*, and *E. moshkovskii*, this is the most common method to diagnose *E. histolytica*.

Diagnosis is best accomplished with a combination of antibody or antigen testing together with microscopic stool examination. Antigen detection is a useful addition to microscopic examination in detecting amoebae, and can distinguish between pathogenic and non-pathogenic strains.[18] Antibody detection is most useful in diagnosing extraintestinal infections when stool examination

is negative. It is worth noting that antibody detection in serum or saliva alone is not enough to indicate acute or current infection. Colonoscopy is not recommended as a routine diagnostic test for amoebiasis because of the high risk of colon perforation during the procedure.

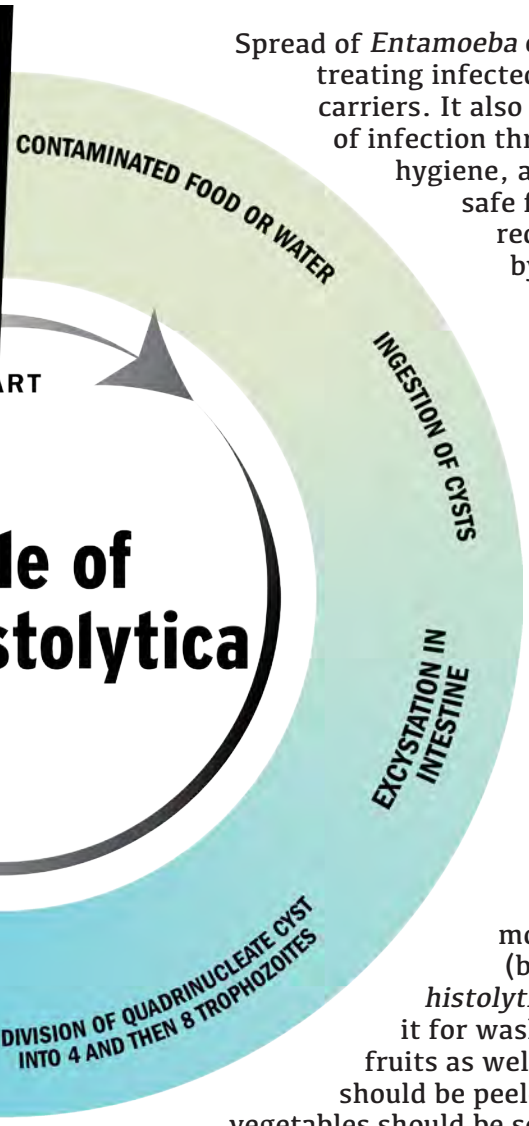


Fig. 2

Spread of *Entamoeba* can be controlled by treating infected and asymptomatic carriers. It also requires prevention of infection through good personal hygiene, adequate sanitation, safe food and water, and reducing transmission by early treatment of carriers. Avoiding sexual practices that involve fecal-oral exposure may reduce transmission of infective cysts. Travelers to endemic areas should avoid untreated water and uncooked foods. Amoebic cysts are resistant to soap and chlorine at the level used in water supplies; therefore, it is advised to boil unbottled water for more than one minute (boiling water kills *E. histolytica* cysts) and to use it for washing vegetables and fruits as well as drinking. Fruits should be peeled and washed, and vegetables should be soaked in vinegar for 10 to 15 minutes before consumption.

Treatment for amoebiasis depends on the strain, severity of symptoms, and site of infection and includes pharmacologic therapy and, in some cases, surgical intervention. Different clinical presentations may favor one approach over the other. Patients with hypovolemia from severe dysentery require IV volume replacement. Liver abscess that is ruptured, or not responding to medical therapy, may be an indication for surgical intervention. The non-pathogenic *E. dispar* and *E. moshkovskii* do not require treatment but should alert the physician that the individual has been exposed to contaminated food or water. In non-endemic areas, even asymptomatic cases of infection should be treated to prevent ongoing shedding of the cyst which may contribute to the spread of infection. This may also help to guard against invasion of the epithelial lining and extraintestinal spread.[16]

For adult patients with mild-to-moderate symptoms, tissue amoebicides (for trophozoites) are recommended:

- **Metronidazole* 500-750mg TID p.o. (12-17mg/kg TID in children) for 7-10 days[19]**
- **Tinidazole* 2g QD p.o. (50mg/kg up to 2g QD po in children > 3) for 3 days[20, 21]**
- **Nitazoxanide* 500mg BID p.o. for 3 days[22]**

For adult patients with severe intestinal and extraintestinal symptoms, metronidazole as described above or a 5-day course of tinidazole may be used initially, followed by a luminal amoebicide to eradicate cysts within the intestinal lumen.[23]

Luminal amoebicides include:

- **Iodoquinol* 650mg TID p.o. (10-13mg/kg TID p.o. up to 2g/day in children) for 20 days**
- **Paromomycin* 8-11mg/kg TID p.o. for 7 days**

None of these drugs should be used during pregnancy.

Adding *Saccharomyces boulardii*, 250mg TID p.o., may decrease the duration of symptoms.[24] Rehydration with fluids and electrolytes is an important component of therapy. People who pass the *E. histolytica* cysts in the absence of symptoms should be treated with a luminal amoebicide. In symptomatic patients, treatment for non-pathogenic *E. dispar* and *E. moshkovskii* is not recommended unless the stool microscopy can not differentiate pathogenic from non-pathogenic forms. While there is some evidence of a transient protective effect provided by mucosal IgA antibodies to *Entamoeba*, further research is needed in pursuit of developing an effective vaccine.[25]

At Diagnos-Techs™ we offer combination testing, including microscopic ova and parasites using 3 stool samples collected at three different times in conjunction with salivary antibody testing for *E. histolytica*, as a part of the Comprehensive O&P Panel (GP9S), or as stand alone tests.

For all medical references please see back for details.

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Clarification:

In ChronoBiology Volume 17, article titled "Blastocystis: A Question of Pathogenicity"

The statement, "In one study, children treated with Saccharomyces cerevisiae had a higher cure rate than those receiving metronidazole." should have read *"S. cerevisiae var. boulardii (S. boulardii)."* Please note that the reference for this statement remains the same. We apologize for any confusion this may have caused.

Article Medical References:

Please see the Newsletter section of our website.

Upcoming Free Webinars:

August 14th, 2014
Adrenal Stress Index: Functional testing & applications

Please visit diagnostechs.com to sign up for free webinars.

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by: John J. White, MD CM

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by: Kamal Henein, MD

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Figure 1: Microscopic view of *E. histolytica* trophozoite. Photo courtesy of CDC website. *http://www.cdc.gov/dpdx/images/intestinalAmebae/Ecoli_troph_tric.jpg*

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Dr. Dialogue

Testosterone:

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