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A Review Of Experience And Update On The Pathology Of Wilson's Disease: Correlations Between Histopathological And Clinico-Pathological Features

Melanie Johncilla

Yale School of Medicine, melanie.johncilla@yale.edu

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A Review of Experience and Update on the Pathology of Wilson's disease: Correlations between

Histopathological and Clinico-pathological features

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Melanie Johncilla

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Abstract

Wilson's disease is a rare autosomal recessive disorder of copper metabolism that often proves a difficult diagnosis for the both the clinician and pathologist largely because of its low incidence and variable presentations. There have been few studies detailing the histopathological features of hepatic specimens in patients with Wilson's disease and fewer correlating these features with clinical presentation. When left untreated, the disease is ultimately fatal, thus good diagnostic criteria that take into account the histopathological features of the disease is important. The aim of this study was to evaluate the histopathological features of hepatectomy and biopsy specimens in patients with Wilson's disease and correlate these features with the clinical parameters of these patients. Twelve biopsy and hepatectomy specimens from patients with Wilson's disease were reviewed and placed into three categories: (A) Near normal/mild, (B) Chronic hepatitis and (C) Acute injury on chronic hepatitis/decompensated cirrhosis. The clinical presentations were reviewed and any correlation between clinical parameters and anatomic features reported. Histopathological features consistent with previous findings in the early stages of Wilson's disease were found in group A with clinical findings in this category of mild elevation of transaminases. In group B, while typical markers of chronic hepatitis were found, glycogenated nuclei, a feature often reported as prominent in specimens with Wilson's disease hepatitis was absent. There was one patient with relevant laboratory data in this group, which was notable for mild elevations of transaminases. In group C, features of acute on chronic injury were found. Regarding clinical parameters, transaminases and bilirubin levels were increased, and alkaline phosphatase:total bilirubin ratio was consistent with previously reported findings of fulminant hepatic failure in Wilson's disease. Urinary copper levels increased with increasing levels of histopathological injury. Staining for copper proved to be an unreliable method of diagnosis. This characterization of the histopathological and clinical features of Wilson's disease further elucidates the challenge of Wilson's disease but also serves to provide some guidance to both clinician and pathologist as to how recognizing the variable presentations of the disease may help in establishing a diagnosis.

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Introduction

Wilson's disease is an autosomal recessive disorder of copper metabolism named for Samuel Kinnier Wilson. He initially described it as progressive lenticular degeneration because of case observations of patients with lenticular nucleus degeneration and cirrhosis of the liver[1]. The disease has a prevalence of 1 in 30,000 worldwide. In some areas, prevalences as high as 1 in 2,600 have been reported. These areas are typically those of high consanguinity[2].

The etiology of Wilson's disease involves greater than 300 mutations[3] in the gene encoding a P type ATPase ATPB7, which is involved in both the incorporation of copper into apoceruloplasmin, a copper carrying protein in the hepatocyte, and into bile for excretion. Malfunction or absence of ATP7B leads to a build up of free (unbound) copper in the brain, cornea and liver. The clinical consequences of this build up are neuropsychiatric (Parkinsonian symptoms, depression, suicidality), ophthalmologic (Kaiser-Fleischer rings i.e. copper build up in the irises of affected individuals) and hepatic (steatosis, cirrhosis, hepatitis).

With early diagnosis and implementation of treatment (primarily chelation therapy), symptom progression and, in some cases, symptom onset can be thwarted. Even though there are regularly updated diagnostic criteria[4], Wilson's disease is known for its high phenotypic and genotypic heterogeneity, which makes it a diagnostic challenge for clinician and pathologist alike. In addition, though early treatises have described the pathology of Wilson disease in detail[5], more recent discourses have not been as comprehensive, nor has there been significant correlation between clinical and pathologic features. The aim of this project was to describe the spectrum of pathological changes of Wilson's disease in our patients with emphasis on correlation with clinical features and diagnostic

parameters.

Wilson's disease mutations and Molecular Pathology

The genetics, molecular pathology and pathophysiology of the disease have been described extensively[6]. In summary, mutations of the Wilson disease gene result in abnormal metabolism of copper. Dietary copper is primarily metabolized by the liver and circulates through the body as ceruloplasmin with help from P type ATPases ATP7A and ATP7B. While both ATP7A and ATP7B are copper transporting proteins, they serve different physiological functions due to the difference of their expression in tissues. ATP7A is primarily localized in the basement membrane of the enterocyte and serves to transport copper into portal circulation. ATP7B, however, is present in the hepatocyte and plays a major role in the incorporation of copper into apoceruloplasmin. Because of their varying roles, mutations in ATP7A and ATP7B inevitably cause different diseases. Mutations in the ATPase ATP7A have been implicated in Menke's disease, an X-linked neurodegenerative disease in which hypocupremia is the main laboratory diagnostic feature. Mutations in ATP7B (located on chromosome 13) are responsible for the development of Wilson's disease.

ATP7B is primarily localized to the trans-Golgi network of the hepatocyte. Copper enters the hepatocyte via a copper transporter Ctr1. The copper chaperone ATOX1 then delivers intracellular copper to ATP7B. There, ATP7B facilitates the binding of copper to apoceruloplasmin and the subsequent secretion of holoceruloplasmin (copper bound to apoceruloplasmin) into plasma. Biliary excretion of copper also involves ATP7B however, there is direct interaction with another protein, COMMD1[6] (previously known as MURR1). The exact function of this ubiquitous protein is yet to be elucidated. However, it is absent in dogs with a non-Wilsonian form of copper toxicosis, Further

investigation showed that COMMD1 is able to bind to ATP7B and aid in the transport of ATP7B bound copper from the trans-Golgi to the bile canaliculus[7].

Ceruloplasmin acts as an oxidase in selected substrate reactions[1]. Interestingly, ceruloplasmin is the major ferroxidase in the body and also aids in iron efflux. The associated disorder of iron metabolism leads to increased liver iron stores so much so that some Wilson's disease patients with hypoceruloplasminemia have been mistakenly diagnosed with hereditary hemochromatosis because of apparent iron overload in the liver[1].

ATP7B is a large 21 exon gene which codes for a 1465 amino acid transmembrane protein. The carrier frequency of the gene for this protein is 1 in 90 while the gene frequency is 0.56%[8]. The most common mutation detailed in Wilson's disease is H1069Q, it is found in 37% to 63% of patients with Wilson's disease of European ancestry[3]. This mutation leads to a failure of trafficking of the protein to its usual position in the trans-Golgi. Other, more recently discovered mutations include R778L (the protein is mislocalized to the endoplasmic reticulum) and P992L found with high frequency in patients with Wilson's disease of Chinese ancestry presenting with primarily hepatic complaints[3]. Most recently, methylenetetrahydrofolate reductase (MTHFR) mutations found in Wilson's disease patients were implicated as a potential cause for the high phenotypic variability of the disease. Patients with particular polymorphisms were less likely than others to exhibit the neurological Wilson's disease phenotype[9].

There have been some studies attempting to detail the genotype-clinical phenotype correlation in Wilson's disease[10,11]. This proves difficult as there are numerous mutations of ATP7B and each mutation is assumed to have significant pleiotropy. In fact, there may be associated mutations in non-ATP7B proteins such as COMMD1, which may cause similar symptoms. Mutations also vary

extensively by population. In screening recommendations for example, in Northern Chinese populations exons 8, 12, 13, 16 and 18 should be screened while exons 5,8, 12, 13 and 16 should be screened in Hong Kong[9]. All of these factors thus limit the clinical application of molecular studies as a primary tool of diagnosis. Identification of unique pathological features in liver biopsies therefore potentially presents a reliable ancillary method of making the diagnosis when fulfillment of diagnostic criteria is unattainable.

Electron Microscopy

Light microscopy of liver biopsies in patients with Wilson's disease has yielded a variety of findings and presents a challenge for the pathologist. In general, features described have included steatosis, glycogenated nuclei and occasional positive staining with rhodanine stains. Published studies on the ultrastructural pathology of Wilson's disease have been limited, partly due to the absence of an electron microscope in most institutions as a standard tool for evaluating pathology specimens, and partly due to the onset of molecular techniques and the readiness with which copper can be quantified in the liver. Other diagnostic tools explored include diagnosis of Wilson's disease by skin biopsy[12]. In a patient presenting with Kayser Fleischer rings and xerosis of the skin, light microscopy of the skin showed hyperkeratosis. Electron microscopy showed dilatation of intercellular space and lysosomes with a heterogenous and granular appearance (incidentally all nonspecific findings of xerosis). However, spectophotometric measurement determined that copper levels in the skin were significantly higher than the control, one of few studies to highlight skin as another potential organ damaged by excess copper.

Transmission electron microscopy along with energy dispersive x-ray spectroscopy have shown

lipofuscin particles containing copper-sulfur (cuprothioneins) and iron phosphorous complexes in the hepatocyte lysosomes of one patient with Wilson's disease[13]. This is considered to be a diagnostic ultrastructural feature. Although other diagnostic features include mitochondrial enlargement and widening of intercristal spaces suggested to be pathognomonic of Wilson's disease, they are unreliable in the setting of cholestasis[13].

Light Microscopy

In early stages of the disease, the damage incurred by copper accumulation has been associated with macrosteatosis, microsteatosis and glycogenated nuclei[14]. Mallory bodies can be seen in up to 50% of biopsy specimens. There are few reports on Wilson's disease patients presenting with acute hepatitis; their biopsies typically show ballooning of hepatocytes, apoptotic bodies, cholestasis and sparse lymphocytic infiltrate[14]. In Acute Liver Failure (ALF) histopathology resembling fulminant hepatitis is typically observed. This is characterized by microvesicular steatosis, marked balloon degeneration, apoptosis, pigment laden Kupffer cells and parenchymal dropout with lobular collapse.

As the disease progresses to cirrhosis, portal inflammation, focal necrosis and glycogenated nuclei are common findings in hepatic specimens. The literature points to steatosis, glycogenated nuclei and the presence of Mallory bodies in periportal liver cells as features used to distinguish the chronic hepatitis of Wilson's disease from other more common etiologies[14]. Autoimmune hepatitis is often mentioned as appearing similar to Wilson's disease. And while lobular necroinflmmatory lesions, ballooning degeneration and interface hepatitis are diagnostic features of both, giant cell change is predominant in the former.

In some cases, cirrhosis develops. A mixed micro/macronodular pattern may be observed although most reports show a macronodular pattern of cirrhosis. Features observed in the chronic hepatitis of Wilson's disease are also observed (i.e. inflammation, Mallory bodies within hepatocytes) after well developed cirrhosis.

Staining

Standard staining is neither sensitive nor specific for the detection of tissue copper. Initially hematoxylin was used for the demonstration of tissue copper. Further investigation into the diagnosis of Wilson's disease via histochemical visualization of tissue copper led to the development of the now commonly used rhodanine and rubeanic acid stains. Other less common stains are: diphenylcarbazide, Waterhouse method, cresyl violet stain, dithizone and Timm's method. The orcein method is the most recent development for detecting tissue copper, it was developed in 1974 and stains metallothioneins (copper-binding proteins).

Consensus on the distribution and staining of copper within the liver in patients with Wilson's disease has not been achieved and remains controversial since there are varying reports in the literature. It has been shown that in the early stages of the disease, copper is distributed throughout the hepatocyte cytoplasm[14]. As the disease progresses, in addition to cytoplasmic distribution, copper is also seen in the lysosomes of hepatocytes and then finally solely in lysosomes in patients with advanced disease. Copper is reportedly rarely seen in the cirrhotic stage of Wilson's disease[14].

Injury progression

In the literature, the typical patient with Wilson's disease will develop cirrhosis in the second decade of life due to copper's damaging effect on liver parenchyma. However, histopathological features consistent with cirrhosis have been found in patients as young as 5 years of age and other changes such as fibrosis and inflammation were found in Wilson's disease patients as young as 4 months and 23 months respectively[10]. Due to these findings and others, there is not any proven relationship between age at diagnosis and progression to cirrhosis. There are however some individuals with primarily neurological manifestations of Wilson's disease who may not progress to cirrhosis. Their liver histology will however may still show some abnormality.

There have been no standardized categories for the hepatic pathological injury progression in Wilson's disease because of its heterogeneity of presentation. Previous reports have categorized the clinical and pathological features in patients with Wilson's disease into: fatty liver, acute hepatitis, resembling autoimmune hepatitis, cirrhosis: compensated or decompensated and acute liver failure[4]. These categories however are not rigid and overlap is inevitable

Clinical Presentation and Parameters

As previously mentioned before, the challenge of Wilson's disease lies in its heterogeneous clinical presentation. It can present predominantly with derangement of liver function, particularly in children. Patients may present as completely asymptomatic and have incidental findings of elevated transaminases, or can present with symptoms resembling acute viral hepatitis, fulminant liver failure and decompensated cirrhosis. Jaundice and hemolytic anemia are also relatively common occurrences in patients with Wilson's disease. Clinical findings – which contribute to the ambiguity of its diagnosis - include elevated gamma-globulin levels and positive ANA and anti-SMA[14].

In the second or third decades of life, neuropsychiatric disease is a more common initial presentation than clinical hepatic disease. 75% of cases present with neurological manifestations and 25% present with both hepatic and neurological presentations after the age of 20[10]. These neuropsychiatric changes include behavioral abnormalities, psychosis and Parkinsonian features (micrographia, tremor, dysarthria and spasticity among others). Less frequently, extrahepatic non-specific features that include renal dysfunction (aminoaciduria), EKG abnormalities and osteoarthritis may be the initial presentation. While neuropsychiatric features are common initial findings, as the disease progresses, the most common finding is predominantly hepatic, varying from fatty change, to hepatitis to cirrhosis[2]. Hepatocellular carcinoma is fairly uncommon in Wilson's Disease although few cases have been reported[14].

The disease is typically diagnosed in patients between the ages of 5 and 40 years old, however molecular studies have found ATPB7 mutations in 70 year old patients[6]. Diagnosis of Wilson's disease has proven difficult despite published diagnostic criteria. The diagnosis typically involves the presence of Kaiser Fleischer (KF) rings, a ceruloplasmin less than 20mg/dl and measurement of liver copper levels. Liver copper levels > 250 micrograms/g dry weight (normal range <35 micrograms/g dry weight) and urinary copper content (24 hr urinary copper levels > 40mcg /24h[4]) support the diagnosis of Wilson's disease . While dry liver copper levels are the gold standard, acquiring a tissue sample from a patient with liver disease carries with it significant risk (bleeding due to coagulopathy, infection etc), thus other more routine chemistries are used in initial investigation. Twenty-four hour urinary copper has also proven beneficial in the diagnosis of Wilson's, however; collection is difficult, particularly in newborns. In addition, in patients who were not suitably educated prior to testing, tap water washing of the urine bottle may contaminate the sample[9].

While the criteria mentioned here have been considered sufficient for the diagnosis of Wilson's disease, there have been numerous reports of patients with overlooked Wilson's disease when they were used, particularly in children. In a recent study of 57 children with a previously received diagnosis of Wilson's disease, KF rings were found in 50% of patients older than 8 years old and in only 7% of patients younger than 8 years of age[10]. Diagnosis in these vague cases was complemented with urinary copper excretion before and after penicillamine (a copper chelator) challenge, copper excretion increased sixteen fold after penicillamine challenge. Liver copper content (the current gold standard) was also used to establish the diagnosis in the absence of more common criteria.

Aminotransferase levels are typically abnormal but have not yet been found to correlate with severity of the disease.   In some studies,the combination of an AST to ALT ratio greater than 2.2 and a Alkaline phosphatase to Bilirubin ratio greater than 4 has been shown to be consistent with Wilson's disease in patients presenting with acute liver failure with a sensitivity that approaches 100%[4].

With the discovery of more ATP7B mutations in Wilson's disease, and the rapid advancement of genetic testing, it is plausible to assume that molecular testing may play a larger role in challenging cases of Wilson's disease. However, due to the rare nature of the disease, routine molecular testing may prove low yield and not cost effective. Nevertheless, it is recommended that such studies only be used in cases in which the diagnosis is difficult even with both clinical and biochemical testing[4]. It must be noted though that most patients with Wilson's disease are compound heterozygotes, thus genetic testing may only prove beneficial in populations with a high incidence of certain mutations such as those populations in the Canary Islands or other such areas with high consanguinity.

Correlations

There are few studies detailing the correlation of histopathological features of Wilson's disease with symptomatology or outcome[10,15]. In one study in children with Wilson's disease, symptomatic patients were more likely to have cirrhosis while asymptomatic patients were more likely to have histological findings consistent with steatosis. Also, in the same study, there were no significant correlations between clinical outcome and histopathological features. In another study in which serial liver biopsies of twelve patients with Wilson's disease were reviewed, it was found that hepatic copper concentration and aminotransferase levels do not correlate with progression of hepatic histopathology[15].

Treatment

Treatment of the disease is most beneficial when diagnosed early in life. Medical therapy is targeted toward reducing the amount of copper accumulations. This can be done with copper chelation (trientine, penicillamine) or zinc, which acts by both reducing the intestinal absorption of copper and inducing the formation of the endogenous copper chelator metalothein. In some cases the copper chelator is combined with zinc as a combined therapy presumably to utilize to methods of increased copper excretion and decreased copper absorption[16].

In a 2010 retrospective study of 288 Wilson's disease patients in Germany, discontinuation of zinc monotherapy and combination therapy occurred with greater frequency than in the cohort on chelator therapy alone. The authors of this study blamed this apparent discontinuation of the high rate of adverse events in the zinc cohort. These events included death and liver transplantation and

concluded that chelating agents proved more successful at preventing hepatic deterioration than zinc. It was recommended that zinc therapy be an alternative for asymptomatic or in patients with solely neurological manifestations of Wilson's disease[16].

Side effects of standard therapy with penicillamine include bone marrow toxicity, elastosis cutis, nephrotoxicity and lupus like syndrome. These severe side effects often lead to noncompliance in roughly a third of patients[16].

Antioxidants such as Vitamin E are sometimes used as adjunctive therapy; its efficacy in decreasing clinical symptoms however is unknown due to a lack of studies. Patients are also advised on low copper diets and avoidance of well water. In the event of acute liver failure however, liver transplantation is both life saving and curative.

Statement of purpose, specific hypothesis and specific aims

Given that the presentation of Wilson's disease was so variable, it was hypothesized that there was a relationship between the pathologic features, the clinical presentation and markers of liver injury (Liver Copper content, 24hr urinary copper, Transaminases, Bilirubin). The objective of this study was to fully characterize the varying pathologic changes in the livers of patients with Wilson's disease and correlate these findings with symptomatology, detailed clinical parameters and clinical outcome.

Materials and Methods

      Pathology specimens from 14 patients with an established diagnosis of Wilson disease were retrieved from the hospital database. Patients were selected based on their fulfillment of either clinical, laboratory or genetic criteria for Wilson disease as well as the availability of surgical pathology material for review. The demographics, clinical presentation, laboratory data, including alanine aminotransferase (ALT), aspartate aminotranserase (AST), alkaline phosphatase (ALK), prothrombin time (PT)/international normalized ratio (INR), total and direct bilirubin (TB/DB), alphafetoprotein (AFP), urine copper (Cu) and ceruloplasmin (CP), imaging, studies and clinical progression were reviewed. Routine H&E, Trichrome, Rhodanine copper and Perl iron stains performed on liver biopsies and/or resections were reviewed and the histological features documented. Pathologic changes were staged using the Batts and Ludwig criteria[17] (0:No fibrosis, 1: Fibrous portal expansion, 2: Periportal or Rare portal- portal septa, 3: Fibrous septa with architectural distortion; no obvious cirrhosis 4: Cirrhosis). Steatosis was characterized as mild (0-30%), moderate (>30% - 60%) and severe (>60%). Iron was graded from 1-4 based on intensity and distribution.

Results

Pt #	Clinical History	Lab Studies AST/ALT	CPL	Urine Cu (mcg/24h)	Liver Cu (mcg/g)	Specimen	Histology
1	54 y/o male presented with Parkinsonian symptoms with significant anxiety and intermittent depression	18, 19: 0.95	5	65	620	Needle biopsy	No significant fibrosis (Stage 0), minimal portal inflammation, no interface hepatitis, patchy balloon degeneration and some glycogenated nuclei. No significant steatosis. Copper and Iron negative
12	51 y/o male					Biopsy	Portal, periportal and focal bridging fibrosis, stage 3. mild to moderate lymohocytic and neutrophilic portal inflammation with mild interface activity. Mild microvesicular steatosis with occasional ballooning degeneration and Mallory. No glycogenated nuclei. Iron negative, copper not done.
3 / 2	14 y/o female presented with acute fulminant hepatic failure [elevated transaminases]	133, 34: 3.9 / 150	7	143	650	Transplant hepatectomy Biopsy	Cirrhosis. Moderate portal lymphoplasmacytic inflammation with moderate to marked interface ductular activity; Moderate steatosis, Mallory hyaline, scattered neutrophilic and lymphocytic lobular inflammation with hydropically degenerated hepatocytes with hepatocellular cholestasis and bile plugs. Occasional acidophilic bodies. Focal copper positivity in periseptal hepatocytes. Iron (1+) positive in occasional hepatocyte and in macrophages ... steatosis with focal
10	7 y/o male evaluated for elevated liver enzymes (incidental	118, 87: 1.36	3			Biopsy	
4	23 y/o male presented with subfulminant hepatic failure [disease]	27, 44: 0.616	7	12,265	1235	Biopsy	Bridging fibrosis, stage 3, marked pericellular fibrosis. Marked portal lymphoplasmacytic, eosininophillic and
11	18 y/o male evaluated for persistently elevated transaminases and low ceruloplasmin	77, 239: 0.32		184	353	Biopsy	neutrophillic inflammation with marked interface inflammatory and ductular activity. Lobular disarray with extensive ballooning degeneration, abundant glycogenated nuclei and rare Mallory hyaline. Hypereosinophilc hepatocytes. Iron and copper negative ... central fibrosis
7	35 y/o female with				600	Biopsy	
5	28 y/o male diagnosed by biopsy after sister was diagnosed with Wilson's	131, 101: 1.3	16	1559	69	Hepatectomy	Cirrhosis, with abundant pericellular fibrosis. Extensive parenchymal loss, with interface ductular activity and marked hepatocellular and cannalicular cholestasis. Extensive ballooning degeneration with moderate lymphoplasmacytic and inflammation and some glycogenated nuclei. Hypereosinophillic hepatocytes. Rare copper, Iron in hepatocytes (2+)
6	19 y/o female presented with decompensation of cirrhosis	47, 36: 1.3	3			Explant	Cirrhosis with parenchymal dropout, marked septal lymphoplasmacytic and some eosinophilic inflammation with ductular proliferation. Mild interface hepatitis. No significant nodular formation,
8	[male with Wilson's disease] presented with decompensated	45, 37: 1.2	4	54	690	Transplant Hepatectomy	mild ductular proliferation. No significant interface hepatitis. Granular eosinophilc

#	Clinical presentation	AST, ALT: ratio		>5000		Sample	Histopathology
6	19 y/o female presented with decompensation of cirrhosis	47, 36: 1.3	3			Explant	Cirrhosis with parenchymal dropout, marked septal lymphoplasmacytic and some eosinophilic inflammation with ductular proliferation. Mild interface hepatitis. No significant nodular inflammation; giant cell change with periportal ballooning degeneration, cholestasis, early Mallory and occasional foam cell appearance. Iron in hepatocytes (3+)
9	17y/o female presented with scleral icterus, lethargy and abnormal liver enzymes	173, 8: 21.6	22	>5000		Explant	Cirrhosis, mild chronic septal inflammation with moderate bile ductular proliferation. Mild to moderate macrovesicular steatosis, extensive ballooning degeneration with focally prominent Mallory hyaline. Marked Hepatocellular and canalicular cholestasis, with focally marked copper accumulation, mild hepatocellular iron.
13	64y/o female with long standing Wilson's disease, presented with acute liver failure	198, 50: 3.96	17			Explant	Cirrhosis. Abundant pericellular fibrosis. Mild lymphoplasmacytic septal inflammation, and ductular proliferation, conspicuous acidophil bodies, marked cholestasis. Extensive ballooning degeneration, cholestasis and occasional periportal Mallory bodies. Copper stains are positive in occasional hepatocytes. Rare iron
14							

#	Group	AST (0-35)	ALT (0-35)	AST/ALT	ALK (30-130)	PT (<14)	INR (0.9-1.4)	Plts	Hbg	Alb	TB (<1.2)	DB (<0.2)	ALK/TB
1	NN/M	18	19	0.9	59	10.9	1	440	14.3	4.3	0.34	0.1	173.5
2		70	150	0.46	NA	NA	NA	NA	NA	NA	NA	NA	NA
10		118	87	1.3	279	10.1	1	408	12.1	4.2	0.23	0.06	1213.0
11		77	239	0.3	122	10.5	0.9	168	15.7	4.7	1.71	0.32	71.3
8	CH/C	45	37	1.2	135	14.9	1.5	71	13.3	3	1.38	0.34	97.8
3	AOCI	133	34	3.9	15	17.9	2.2	197	4.5	3.4	10.5	6.56	1.4
4		27	44	0.6	163	13.6	1.2	75	14	4.7	0.93	0.18	175.3
5		131	101	1.3	147	22	2.2	159	6.2	2.4	8.64	5.45	17.0
6		47	36	1.3	99	21.9	3.6	67	10.1	3	8.16	3.98	12.1
9		173	8	21.6	2	29.6	2.9	146	9.5	2.3	47.8	39.5	0.0
13		198	50	3.9	162	35.6	8.8	158	11.4	2	22.7	17	7.1
AVG		94.2	73.2	3.6	118	18.7	2.5	189	11.1	3.4	10.2	7.4	11.6
Sd		60.2	67	68.7	80.9	8.6	2.4	132	3.6	1	15	12.5	5.4

Table 1. Available demographic data and histopathology for all patients with Wilson's disease.
Table 2. Available clinical/laboratory data. NN/M (Near normal/Mild), CH/C (Chronic Hepatitis/Cirrhosis), AOCI (Acute on Chronic Injury)

Demographics, specimens, histopathology, clinical and laboratory data are presented in Tables 1 and 2. Cases highlighted in green correspond to the near normal/mild category; cases in blue correspond to the chronic hepatitis category while cases in red correspond to the acute on chronic injury category.

Demographics

Of the cases reviewed, there were 8 males and 6 females of age ranges 7 – 64 years at time of biopsy/resection, with a mean age of 31 +.18 years. 2 Patients were Caucasian, 2 were Asian, 1 was Black, 2 were Hispanic, 2 unknown and 4 of the patients listed their races as "other".

Clinical Presentation

All but one patient was diagnosed based on an initial presentation of symptoms related to or with incidentally discovered deranged liver. Presentations ranged from acute liver failure to incidentally elevated transaminases in a virtually asymptomatic patient. The exception was a 54-year-old man who presented primarily with neuropsychiatric symptoms, which included Parkinsonian features, significant anxiety and intermittent depression. He had no abnormalities of liver enzymes, however, was found to have a low serum ceruloplasmin. Diagnosis of Wilson disease was confirmed by positive gene studies in this patient.

Laboratory values

Ceruloplasmin was available in 8 patients and ranged from 3-22U/L with a mean of 9+.7U/L.

One patient had a normal ceruloplasmin at 22U/L, in this case the diagnosis was based on histopathology findings and a KF ring on exam. AFP, which was also available in 8 patients, was elevated in 4 patients (range 3 to 40U/L, mean 16+.16U/L). None of these patients however developed Hepatocellular Carcinoma.

Eight of ten patients with available data had elevated AST levels (range 18 to 198U/L, mean 97+.62U/L) while seven of ten patients had elevated ALT levels (range 8 to 239U/L, mean 65+.67U/L). The AST/ALT levels were > 2.2 in two patients who presented with acute liver failure. ALK levels were elevated in five patients (range 2 to 279U/L, mean 118+.80U/L). TB values were elevated in seven of ten patients (range 0.34 to 47.8U/L, mean 10.2+.14.9U/L) while DB values were also elevated in seven patients (range 0.06 to 39.5U/L, mean 7.3+.12.5U/L). ALK:TB ratio was less than 4 in only 2 patients (mean ratio 11.6+5.4).

Dry Liver Copper values were available for seven patients. Six of the seven values were well above the suggested 250mcg/g (mean 603 + 355mcg/g) cut off for Wilson's disease. There was one patient in the 'Acute on Chronic Injury' category with a liver copper value of 69 mcg/g. There was no observed correlation between these values and the level of histopathological injury.

Urinary Copper values were available for only six patients. All values were well above the suggested 20mcg/24h (mean 2378+ 4878mcg/24h). Values increased with increasing levels of hepatic injury (Table 1).

Most patients presented with a coagulopathy, thrombocytopenia and anemia (mean INR 2.5+.2.4U/L, platelets of 188+.132 x10^3/uL and Hemoglobin (Hb) of 11+.4U/L (Table 2).

Imaging

Right upper quadrant ultrasounds were reported on all patients. The most commonly reported liver abnormalities were hepatomegaly, increased echogenicity, heterogeneity and nodularity consistent with cirrhosis. The impression on imaging correlated with biopsy findings in all but one patient, in whom there was significant steatosis but an unremarkable scan.

Histopathology

Patients were placed into three groups based on their pattern of histopathological liver injury and clinical correlate: Near normal/mild liver injury, chronic hepatitis (which included chronic hepatitis with or without cirrhosis), acute on chronic hepatitis and decompensated cirrhosis. Near normal was defined as grade 0-1 fibrosis, chronic hepatitis[18] as hepatocellular necroinflammatory lesions with parenchymal dropout, lymphocytic inflammation and greater than grade 1 fibrosis, cirrhosis as defined nodular fibrosis and acute on chronic injury was defined as lobular disarray or other acute changes on a background of chronic hepatitis/cirrhosis.

General observations

Typical findings associated with Wilson disease were seen. These included glycogenated nuclei, steatosis and Mallory bodies. Cases that were devoid of steatosis were cirrhotic (5 and 6) and the most marked steatosis was seen in samples with minimal fibrosis. Hepatocyte hypereosinophila of unclear significance was observed in two cases.

Near normal/Mild

There were four patients in the "Near normal/mild" category. While the fibrosis grades were 0-1, the steatosis grades ranged from 0 – 3. Biopsies in this category were significant for mild portal fibrosis in three of the four cases; architecture was preserved in all cases (Figure 1A). There was an absence of interface hepatitis, lobular inflammation, Mallory hyaline, or copper accumulation by staining. Glycogenated nuclei were seen in all cases (Figure 1B), as was patchy ballooning degeneration. All patients were male and ranged in age from 7 -54 years. One patient presented with non-hepatic complaints while the others were noted to have asymptomatic persistent increases in transaminases, the most prominently elevated occurring in a patient with Stage 1 fibrosis. Bilirubin was normal in all patients while ALK was elevated in three of the four patients. One patient, an 18 year old man, had a more marked elevation in ALT than AST, which is not typically seen in Wilson disease. Hepatomegaly was the only ultrasonographic finding of significance in this group.
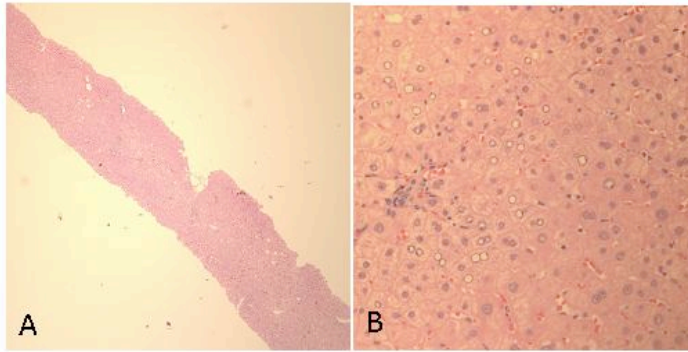
Figure 1. Near Normal/Mild
A (H&E): Architecture is preserved with only mild steatosis and minimal inflammation
B (H&E): Abundant glycogenated nuclei scattered throughout hepatic parenchyma

Below are details of patients in this category

Patient #1 was a 54 y/o male who presented with predominantly neuropsychiatric complaints; there was no steatosis in his biopsy. However glycogenated nuclei and mild ballooning degeneration were observed.

Patient #2 was a 37y/o male evaluated for elevated transaminases. His biopsy was significant for moderate microvesicular steatosis and glycogenated nuclei.

Patient #10 is a 7 year old male who presented with an initial diagnosis of Kawasaki's disease (strawberry tongue, fevers). Upon further testing, abnormal liver enzymes were noted and the patient

underwent work up for liver disease. AST, ALT were elevated at presentation, Ceruloplasmin was low

at 3. Copper and iron stains were negative. His biopsy was consistent with stage 3 steatosis, but grade

1 fibrosis. There were few glycogenated nuclei and minimal portal inflammation.

Patient #11 was an 18 year old male who presented asymptomatically with persistent elevations

in transaminases. His AST and ALT were elevated (notably, this patient had a much more elevated

ALT than AST, not typically seen in Wilson's disease). All other lab values were within normal limits.

Histopathology of the biopsy specimen revealed negative copper and iron staining, grade 3 steatosis

and grade 1 fibrosis. Abundant glycogenated nuclei were seen as well as minimal portal inflammation

and ballooning degeneration of cells.

Chronic hepatitis

There were two patients with bridging fibrosis and one with cirrhosis in this group (Figure 2A).

Sinusoidal fibrosis was also prominent in one of these cases. All three cases were devoid of significant

acute changes with mild to moderate portal inflammation, none to mild interface inflammatory activity

and only patchy ballooning degeneration and occasional acidophil bodies, (Figure 2.). Neither of the

two cases with bridging fibrosis had copper or iron deposition and steatosis was mild to focally

moderate. The cirrhotic patient had rare Mallory hyaline and copper in rare periportal hepatocytes

(Figure 2B). Steatosis was minimal in this group.

Of the patients with bridging fibrosis, one patient was male, the other female. No laboratory

data is available on either patient. The cirrhotic patient presented with features of decompensated
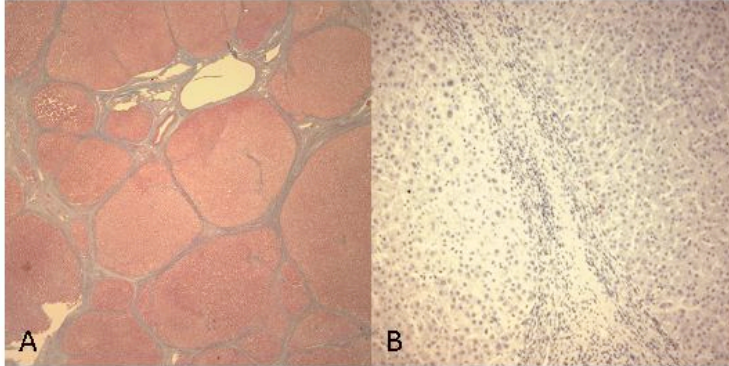
cirrhosis.

Figure 2. Chronic Hepatitis
A (Trichome): stain showing mixed but predominantly macronodular cirrhosis.
B (Rhodanine stain): Rare copper seen near fibrous septa

Below are details of patients in this category

Patient #7 was a 35y/o female with long standing Wilson's disease. Biopsy report showed sinusoidal fibrosis (periportal and pericentral) with focal bridging but without cirrhosis. Lobular disarray was noted. Fibrosis grade 3, Steatosis grade 2. Mild to moderate chronic inflammation was noted with mild interface hepatitis and absent cholestasis or Mallory bodies. Copper and iron stain was negative.

Patient #8 was a 50y/o female who was living with Wilson's since the age of 8 and on zinc therapy; she had an orthotopic liver transplant in 2008. Her labs were notable for mild elevations in transaminases and bilirubin as well as low platelets on presentation. Her biopsy was significant for well established macronodular cirrhosis and mild to moderate lymphoplasmacytic infiltrates as well as ductular proliferation. There was however no significant steatosis or glycogenated nuclei in this patient.

Patient #12 was a 51y/o male with Wilson's disease; there were no laboratory data available. However, his histopathology revealed portal, periportal and bridging fibrosis, mild to moderate lymphocytic and neutrophillic infiltrate. No glycogenated nuclei were seen. Grade 1 steatosis and few Mallory bodies

Acute on Chronic Injury

This represented the major pattern seen in this review. There were seven patients in the "Acute on Chronic Injury" category. There were six cases with cirrhosis and one with bridging fibrosis. Typical markers of acute hepatic injury (i.e. parenchymal dropout, necroinflammatory activity,) were observed in all specimens (Figure 3. A-D). More specifically, biopsies and liver explants were significant for lobular, portal and septal inflammation of varying degrees and lobular disarray was a consistent feature. The inflammatory infiltrate was primarily lymphocytic although eosiniphillic and neutrophillic inflammation was seen in a minority of the specimens. Moderate to marked periportal lymphoplasmacytic inflammation was observed in three of the specimens while mild to moderate chronic septal lymphoplasmacytic inflammation was observed in the other four cases. Neutrophillic inflammation was seen in only two of the seven cases. Mild to marked interface hepatitis and ductular

activity was present in four of the seven cases. Extensive ballooning degeneration, glycogenated nuclei and acidophil bodies were present in all of the cases while Mallory bodies and cholestasis were observed in six of the seven specimens. Steatosis was seen in three of the seven cases, with grades of 1- 2. Copper staining was positive in five of the seven samples, staining ranged from diffusely positive to occasional hepatocyte staining (Figure 4A, B). Iron staining was strongly positive (2+, 3+) in two cases (Figure 4C, D)

In terms of clinical presentation, patients in this group ranged in age 13-64y/o, with a mean age of 25. There were four females and three males. All patients presented with fulminant, subfulminant hepatic failure or decompensated cirrhosis. Other significant clinical findings in this group included coagulopathy and varying degrees of cytopenias. Transaminases were available for six of the seven patients. AST levels were elevated in all but one patient. ALT levels were also elevated in six of seven patients. AST/ALT values were greater than 2.2 in 3 of 7 patients in this category. Ceruloplasmin was decreased in five of the six patients. TB was elevated in all patients while DB was elevated in five of six of the patients. ALK in this group ranged from 2-163U/L, three patients presented with elevated values. In previous studies, patients with Wilson's disease presenting with fulminant hepatic failure typically have ALK/TB ratios far less than 4[4]. ALK/TB values were less than 4 in only two patients presenting in this category (#3 and #9). There were only two patients with available dry copper values in this category and one patient (#5) had a value below the suggested criteria for Wilson's disease. Interestingly, urine copper levels in this category were well above suggested criteria ranging from 1559mcg/24h to 12,265mcg/24h. Common ultrasonographic findings included shrunken heterogeneous hyperechoic livers consistent with cirrhosis.
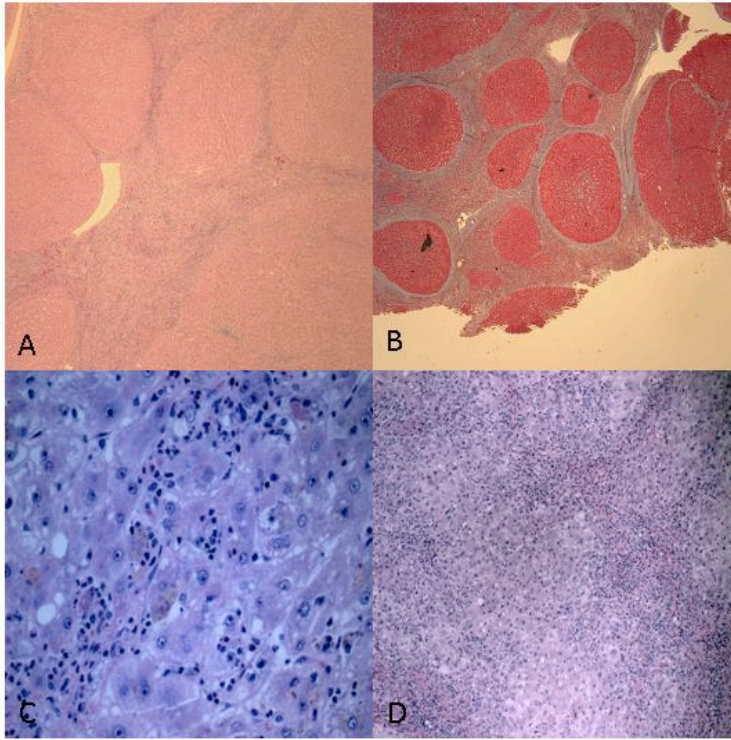
Figure 3. Acute on Chronic Injury
A (H&E): and B (Trichome stains) showing mixed nodular cirrhosis and septal inflammation.
C (H&E): Ballooning degeneration with prominent Mallory hyaline
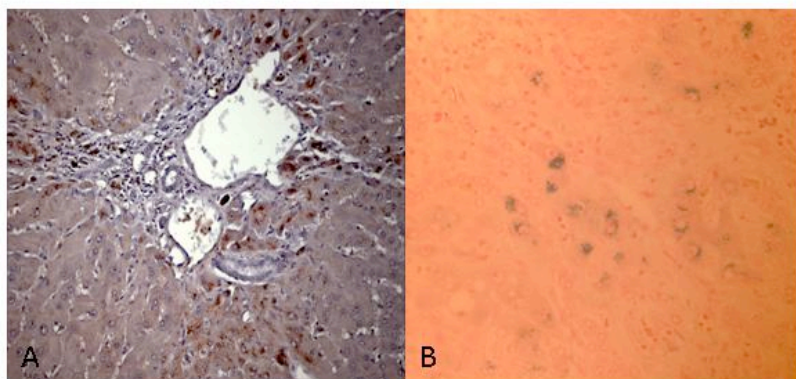D (H&E): Lymphoplasmaccytic inflammation with periseptal lobular disarray

Figure 4. Acute on Chronic Injury - Iron and Copper Staining
A (Rhodanine stain): Periportal copper
B (Perl stain): Diffuse iron

Below are details of patients in this category

Patient #3 was a 14 year old female admitted in acute fulminant hepatic failure. Pathology was notable for parenchymal loss, lymphaplasmacytic inflammation, moderate ductular proliferation and periseptal acidophil bodies. In this case, Copper stains were variable but most prominent in periseptal hepatocytes. In terms of other staining, iron was 1+ in macrophages and in periportal areas. The steatosis grade was 1, with fibrosis grade 4. AST levels were elevated in this patient, ALT levels remained normal. Chemistries were also notable for hyperbilirubinemia and anemia.

Patient #4 is a 23 year old male presenting with epigastric abdominal pain diagnosed as

'subfulminant hepatic failure', Liver enzymes were mildly elevated. Glycogenated nuclei were present in his biopsy as was lymphoplasmacytic and eosinophillic portal inflammation and ballooning degeneration. There was marked interface and ductular activity and particularly eosinophillic hepatocytes. Copper and iron stains were negative. Fibrosis grade was 3, however there was no steatosis.

Patient #5 was a 28 year old male with elevated transaminases and hyperbilirubinemia whose hepatectomy specimen was noticeable for glycogenated nuclei, extensive parenchymal loss and marked cholestasis. The copper staining on this specimen was rare. Iron stains were negative. Fibrosis grade 4, Steatosis grade zero. There were also hypereosinophillic hepatocytes in this case along with intranodular lymphoplasmocytic inflammation. This patient had a dry copper level of 69 mcg/g however his urine copper values were 1559mcg/g.

Patient #6 was a 19 year old female who presented from Puerto Rico with rash and abnormal liver enzymes, further investigation revealed Wilson's. Marked portal inflammation, lymphoplasmacytic inflammation, cholestasis and early Mallory bodies. Copper and Iron stains were positive (with Copper being localized to periportal areas) and seen more prominently within larger nodules. Steatosis and glycogenated nuclei was absent in this case. Patient had very mildly elevated liver enzymes and hyperbilirubinemia.

Patient #9 was a 17 year old presented with rash and epigastric pain with abnormal liver enzymes on presentation. This patient had a normal ceruloplasmin (22mmol/L). Her AST was elevated but her ALT was low normal. Her alkaline phosphatase was also decreased at 2mmol/L. She was also noted to have significant hyperbilirubinemia and mild coagulopathy. Pathology was

significant for grade 2 steatosis, grade 4 fibrosis (cirrhosis) and positive iron and copper staining. There were focally abundant Mallory hyaline. Mild to moderate chronic inflammation with cholestasis and regenerative nodules were also noted.

Patient #13 was a 64 year old female with long standing Wilson's disease. She had been treated with penicillamine and was switched to zinc because of poor tolerance of the former. The patient eventually had an OLT in 2007 due to acute liver failure. AST and ALT were elevated (AST/ALT>2). Notably in this patient's labs were an increased total bilirubin with normal direct (perhaps due to hemolytic anemia seen in Wilson's patients). Histopathology revealed grade 2 steatosis and grade 4 fibrosis with iron and copper stains both being positive in occasional hepatocytes. There was mild septal inflammation, marked cholestasis, glycogenated nuclei and some Mallory bodies.

Patient #14 is a 13y/o male received as a consult whose biopsy was negative for both iron and copper. Significant findings in the specimen included mild steatosis and grade 3 bridging fibrosis.

Discussion

As demonstrated in this review, both the anatomic and clinical presentations of Wilson's disease are heterogeneous. Its course is also affected by timing and tolerance of treatment. As demonstrated in prior studies, the earlier the treatment, the better the outcome. In this study, we aimed to first categorize these patients on the basis of histopathological injury of their biopsies and hepatectomies. The pathology and clinical parameters were then detailed and attempts were made at correlation.

Our review supports the pathology of the early stages of Wilson disease as represented by steatosis and glycogenated nuclei, often without accumulation of copper. The most consistent finding in this group of near normal liver biopsies is glycogenated nuclei. The pathogenesis of these glycogenated nuclei is largely unknown, however, it has been described to be due to complex sugars that deposit in the nuclei of the hepatocyte. Glycogenated nuclei have been seen in NASH (Non-Alcoholic Steatohepatitis), Diabetes Mellitus and Wilson's disease years before symptoms begin[14]. It is however presumed that this fairly nonspecific finding may represent very early hepatic injury even though glycogenated nuclei have been observed in biopsies of healthy individuals[14].

Interestingly, with worsening of histopathological injury from near normal to definite chronic hepatitis to acute on chronic injury, the findings found in this review were not entirely consistent with those detailed before[14]. This provides further evidence of the difficulty of the diagnosis of Wilson's disease for the pathologist. In the 'Chronic Hepatitis NOS' category, for example, lymphoplasmacytic inflammation has been suggested as a feature to distinguish Wilson's disease hepatitis from hepatitides of other etiologies[14] but is clearly not a specific enough change to utilize in this setting. It is important

to note however that the previously ubiquitous glycogenated nuclei found in early stages of the disease were notably absent in cases of chronic hepatitis and compensated cirrhosis. In addition unlike previous reports, copper staining was rare if not absent in most of these cases.

Oddly, as the disease appears to progress, either as decompensated cirrhosis or acute injury on chronic hepatitis, abundant glycogenated nuclei are seen. Of note, in this category, there was also one case with giant cell change, a finding typically seen in autoimmune hepatitis. This patient however did not fulfill diagnostic criteria of autoimmune hepatitis.

Another significant observation is the overlap between acute on chronic liver injury in Wilson's disease and other features such as cholestasis and iron overload. In particular, since ultrastructural features may overlap with those of other cholestatic disorders, ultrastractural analysis may have limited application in patients with acute liver injury and cholestasis.

Diagnosis: Anatomic and Clinical pathology correlations

Early diagnosis of Wilson's disease is necessary, as treatment with copper chelators has been proven to delay the subsequent hepatic and lenticular degeneration cause by copper buildup. Thus thorough characterization of patients presenting in this review's "Near Normal/Mild" category is essential. As seen above, cases in this category were significant for mild elevations in transaminases on clinical presentation and steatosis and glycogenated nuclei on biopsy. Presented as such, this perhaps generates the largest differential for the pathologist without any further molecular or genetic studies given that in all four cases iron and copper staining were negative. The differential may include

any of the far more common causes of mild chronic hepatitis (autoimmune, viral, toxin). Regarding

trends of clinical parameters in this group, there were varying elevations in transaminases (mostly

AST>ALT as seen in previous reports of Wilson's disease). Importantly, transaminases were not as

high as the levels seen in the viral hepatitides (AST and ALT levels are often 5 – 10 times the upper

level of normal) nor were AST/ALT ratios greater than two as typically seen in alcoholic hepatitis.

Dry copper levels were also elevated. However, Total and Direct Bilirubin were normal in all cases but

one. Here, as mentioned in previous studies, copper quantization proves to be the only definite clinical

lab value in diagnosing liver disease. It is therefore imperative that when this minimal degree of liver

changes are noted, if a suspicion exists for Wilson disease based on other parameters, liver quantization

is performed.

In the subsequent groups representing patients with increasing hepatic injury due to Wilson's

disease, clinical parameters (transaminases) increased with hepatic injury but were not consistent with

previous reports. AST/ALT ratios in the acute on chronic category were greater than 2.2 in only two

patients, the same number had ALK/TB ratios less than 4. This is unlike previous studies which have

shown that the combination of AST/ALT > 2.2 and ALK/TB > 4 is consistent with Wilson's disease in

patients presenting with acute liver failure. This is a retrospective study and it is important to note that

some of the patients at Yale New Haven Hospital were already on treatment at the time of their

presentation, this may have skewed the results. Also, such differences in transaminase and bilirubin

levels illustrate the unreliability of solely clinical parameters in diagnosing Wilson's disease.

While dry copper levels did not increase with increasing levels of hepatic injury, urine copper

levels – in those patients with available data – did. This finding may prove important in diagnosing

and following patients with Wilson's disease. Dry copper levels have been the gold standard of

diagnosis but this study shows a patient with Wilson's disease with a dry copper level of 69mcg/g, well under the suggested criteria of 250mcg/g. His urine however surpassed the cut-off and diagnosis was confirmed. Apart from illustrating the difficulty of diagnosis, this finding shows urine copper to perhaps be more reliable in assessing not only the presence but the severity of Wilson's disease. There are however difficulties with collection of 24h urine samples and many opportunities for contamination.

This study replicates the often-repeated observation that a negative liver copper stain does not exclude Wilson disease. Previous investigation into Timm's silver stain (not used in this study) found it to be sensitive to the cytosolic distribution of copper in the early stages of Wilson's disease. The standard rhodanine and rubeanic stains are often negative at these stages[19]. In a study to assess the sensitivity of the three most commonly used stains (Timm's, rhodanine and orcein), 79 liver biopsies from patients with Wilson's disease were categorized into three different groups: steatosis, interface hepatitis and chronic hepatitis/cirrhosis. Timm's s silver stain was found to be more sensitive in its detection of copper in all three groups. This study also demonstrated the unreliability of histochemical detection of copper in Wilson's disease. Timm's silver stain, for example, only stained 58.6% of biopsies with chronic hepatitis/cirrhosis, 40.1% of biopsies with interface hepatitis and 30.4% with steatosis. It has been suggested that Timm's be used in referred cases with limited tissue available for staining[19].

In addition to emphasizing the fallibility of the copper stain, this study shows the absence of any correlation between copper staining and dry copper levels. In fact, positive copper and iron stains seemed to correlate more with increasing tissue injury than actual copper levels. Patient #5 – in the acute on chronic injury category - for example had a dry copper level of 69mcg/g and a positive copper

stain, while Patient #1 – in the near normal/mild category – had a dry copper level of 650mcg/g with a negative copper stain. This difference may be because rhodanine is less likely to stain the abundant cytoplasmic copper present in early stages of the disease and may be more apt to stain lysosomal copper present in advanced stages of the disease.

Interestingly also, only a minority of patients have bland chronic hepatitis, with or without cirrhosis. The vast majority of cases reported here show some degree of acute liver injury, exemplified by any combination of ballooning degeneration, apoptosis, parenchymal dropout with lobular collapse. In addition, as has been observed, in these cases with acute injury, cholestasis is a prominent feature, as is some measure of disordered iron metabolism and increased iron.

The iron overload noted in the specimens in the acute on chronic liver injury category can be due to the intravascular hemolysis seen in some reported cases of Wilson's disease, unfortunately there were no serum iron levels to correlate with the histopathological findings. The suggested cause of this hemolytic anemia is the increased oxidative stress caused by copper accumulation within the cells[20].

The iron overload could also be due to the hypoceruloplasminemia (and potentially, the hypocupremia) in these patients. As mentioned before though not completely characterized, ceruloplasmin is a ferroxidase essential for iron mobilization and transport thus it can be assumed that the decreased ceruloplasmin seen in Wilson's disease patients would lead to a build up of iron in major organs of the body and an absence thereof in the serum. Aceruloplasminemia, an autosomal recessive disease caused by loss of function mutations in the ceruloplasmin gene, illustrates the effect of low to absent ceruloplasmin on iron metabolism. In this case, iron mobilization is severely impaired and builds up in the pancreas, liver and brain. Hypoceruloplasminemia does not wholly explain iron

overload however as iron staining was positive in one of the reviewed cases, this patient had a

ceruloplasmin of 22U/L. It is important to note though that ceruloplasmin is an acute phase reactant,

thus high levels are to be expected in patients with acute on chronic injury.

Nevertheless, iron proves to be an important marker of liver injury in the context of Wilson's

disease as copper has been previously found to play a crucial role in its metabolism not only in the

context of ceruloplasmin but also in Cu dependent ATPases. The ATP7A/ATP7B homologue found in

Saccharomyces cervisae yeast Ccc2p has been characterized in previous studies as a copper dependent

ATPase necessary for iron uptake[21]. In future studies, in addition to the clinical parameters detailed

above, it may be worthwhile to correlate dry iron levels with disease states in order to further detail

diagnostic criteria, and to determine whether liver iron levels may be a useful marker of acute liver

injury in the setting of Wilson disease.

Because of the relative recent incorporation of molecular diagnostics into clinical practice and

the rare nature of Wilson's disease, it is important to note that although Wilson's disease is

characterized by a mutation in ATP7B, other mutations in other copper dependent ATPases or copper

dependent proteins may be implicated in this disease of copper accumulation. These varying mutations

may potentially account for both the varied clinical presentations and varied patterns of

histopathological injury seen above.

Previous reports[4] have categorized the clinical and pathological features in patients with Wilson

disease into: fatty liver, acute hepatitis, resembling autoimmune hepatitis, cirrhosis: compensated or

decompensated and acute liver failure. In this series, none of these patients with acute liver failure was

without cirrhosis thus it may be possible that in some settings, such acute liver failure occurs only in

the present of extensive prolonged liver damage.

In this review, a clinical presentation of acute liver injury correlates with the histological features though not entirely consistent with previous reports. Correlation between other histological features and clinical parameters is less clear, though some trends were noted. As seen in this study, despite varying levels, the ceruloplasmin remained low, if not low normal in most cases. Though copper staining has proved an unreliable method for initial diagnosis, the most severe cases with acute injury were more likely to stain positively with copper and therefore, unlike ceruloplasmin, may possibly correlate with severity of acute injury. In addition, and perhaps most important, urine copper appears to correlate closely with hepatic injury and was positive in a case of Wilson's disease in which dry liver copper was negative (according to diagnostic criteria). Thus, this may prove beneficial in diagnosing and trending of disease although further studies are warranted. Testing urinary copper also employs a far less invasive (and presumably safer) method of testing. Both patient and physician should be thoroughly educated on 24h urine collection (avoidance of tap water and Foley catheters, usage of pediatric urine bags that do not contain copper) so as to minimize contamination.

The characterization of Wilson disease still remains a challenge and while advances in molecular techniques may theoretically be able to facilitate diagnosis, these may prove prohibitively expensive given the increasing number of ATP7B mutations and the lack of prevalence of the disease. A thorough awareness of clinical presentation, varying histopathological features and clinical parameters that provide hints to both disease process and severity is therefore be useful in the clinical management and diagnosis of Wilson's disease.

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