VIDAS TEST OF IgG AVIDITY FOR DETECTION OF ACUTE TOXOPLASMOSIS IN THE EARLY PREGNANCY

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Abstract

Toxoplasmosis is usually asymptomatic, but can have severe consequences if it occurs in immunodeficient subject or fetuses. The diagnosis of toxoplasmosis during pregnancy is often based on maternal serological testing for IgM and IgG anti-Toxoplasma antibodies. Persistence of IgM for long periods, posses' problems in distinguishing acute from chronic infection. The evaluation of specific IgG avidity enables more accurate dating, since avidity rises progressively during the course of infection.

Seventy six women in the first 16 weeks of pregnancy were screened for VIDAS IgM, IgG antibodies and VIDAS toxo-IgG avidity. Low avidity antibodies were demonstrated in 2 (33.3%) of 6 sera positive with IgM assay and 4 (12.12%) of sera positive with IgG assay. Low avidity also detected in 2 (3.27%) of 61 sera negative with IgM. The low avidity suggesting a recent infection, while high avidity in 3 (50%) of the 6 positive IgM and 24 (72.72%) of 33 positive IgG indicating that the infection acquired in the distant past. These findings highlight the value of VIDAS IgG avidity when used in combination with the VIDAS IgM and IgG assay to provide a confirmatory evidence of an acute infection with a single serum specimen for pregnant women.

اختبار VIDAS لالفة IgG في تشخيص داء المقوسات الكوندية الحاد في الحمل المبكر

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الخلاصه

داء المقوسات الكونديه مرض لا تظهر اعراضه ولكن له تبعات خطيره اذا اصاب مرضى نقص المناعه او الاجنه. ان تشخيص داء المقوسات الكونديه خلال الحمل يعتمد غالبا على الفحص المصلي للام لكل من الاجسام المضاده IgG,IgM النوعيه المضاده للمقوسات. ان ثبات الIgM لفترات طويله تسبب مشاكل في التمييز بين الاصابات الحاده والمزمنه. ان تقييم الالفه النوعيه للIgG تساعد في تحديد دقيق لزمن الاصابه وذلك لان الالفه تزداد بشكل تدريجي خلال مراحل الاصابة.

سته وسبعون امراة حامل في الاسبوع السادس عشر اجري لهن فحص الاجسام المضادة IgG و IgM بطريقة VIDAS و الالفة لل IgG بطريقة ال VIDAS ايضا. لقد وجدت الفة واطئة للاجسام المضادة IgG في 2 (33.3%) من المصول الموجبة لفحص IgM و 4 (12.12%) من المصول الموجبة لفحص IgG . كما وقد وجدت الفة واطئة لل IgG في 2 (3.27%) من 61 مصل سالب لفحص IgM.

ان الالفة الواطئة لل IgG تشير الى اصابة حديثة بينما الالفة العالية لـ 3 (50%) من 6 حالات موجبة لفحص IgM و24 (72.72%) من 33 حالة موجبة لفحص IgG تشير الى ان الاصابة قد حصلت منذ زمن بعيد. هذه النتائج تبرز قيمة فحص الالفة للـ IgG نوع VIDAS عندما يستخدم بالتازر مع فحص VIDAS للاجسام المضادة IgG وIgM للحصول على اثباتات تاكيديه للاصابه الحاده باستخدام عينه مصليه مفرده للنساء الحوامل.

Introduction

Phenoxazine was first made by Bernthsen in 1887 different C and N substituted phenoxazines were found to serve as potent dyestuffs. Oxazine dyes are derivatives of phenoxazine which is widely used in biological stains [1-3]. These have been studied for staining brain tumors and as tuberclostatic agent [4-6].

In general the reaction of phenoxazine dyes have been claimed to posses a wide spectrum of biological and pharmacological activities.

Certain derivatives (Namely isopheno- xazines) have been isolated from plant animal sources [8], and till the last decade, little was known about the metabolism of phenoxazine in biological systems [9].

Phenoxazine compounds like antinomycin and questionmycin have been studies, and the first represents group of antibiotic activities used as potent closely related compound possessing anticancer activity [10-14].

Experimental

Melting points were recorded using Gallen-Kamp melting point apparatus and are uncorrected. FTIR spectra were recorded using KBr disk on Shimadzu Fourior transform infrared spectrometer FTIR-8400s.U.V spectra were recorded on a U.V-Visible spectrometer (Shimadzu) U.V 160A.

1HNMR spectra were recorded on Shimadzu FT - NMR 300 MHz with tetramethyl silane as internal standard. Thin Layer Chromatography (T L C) was preformed on aluminum sheets precoated with silica- gel (F.254).

General procedure for the preparation of compounds Phenoxazine(1)[15]

A mixture of (109 g, 1 mole) of o-aminophenol,(2g) $ZnCl_2$ and 5ml H_3PO_4 was heated in a sand bath maintained at 270-275C^0 for 4 hrs. The reaction mixture was cooled and extracted with cyclohexane in a soxhlet extraction apparatus. The a solvent was removed and the formed colorless needles were crystallized from ethanol m.p.152-154C^0, yield (54%) IR 3405 cm^{-1} (NH str.).

10-acetyl phenoxazine(2)[16]

A mixture of (40g, 0.22 mole) of phenoxazine, 140 ml acetic anhydrides and 109 ml, glacial acetic acid was refluxed for 2 hr, cooled to room temperature and diluted with 150 ml cold water to give colorless prisms. The product was recrystallized from ethanol, m.p. 142C^0 yiled (38-36.78%) IR 1669 cm^{-1} (C=Ostr.).

Pt I: 10-(oxo alken-1-yl) phenoxazine derivatives (3a-3i)

A mixture of (2g,0.01 mole) of 10-acetyl phenoxazine and 0.012 mole of the appropriate aldehyde in 80 ml, of ethanol and (1.5 ml) of 1 % NaOH in ethanol was refluxed for 2 hr. The reaction mixture was poured in cold water. The precipitate was filtered off and recrystallized from ethanol.

Table (I) represents, the physical data of compounds [3a-3i].

Pt II: 10-(1- acetyl pyrazolin-3-yl)phenoxazine derivatives (4)

To a solution of 10- (3- phenyl oxopropen-1-yl) phenoxazine (3) (0.313g, 0.001 mole) in acetic acid (96%, 1ml) hydrazine hydrate (0.4 ml) was added and the mixture was refluxed for 5hr. The product separated out on cooling was crystallized from ethanol. FTIR of this group of compounds showed absorption bands at (1570-1595) cm^{-1} (C=C str.), and 1590-1618 cm^{-1} (C=N str.) (Table2).

Pt III: 10-(1-phenyl pyrazolin-3-yl) phenoxazine derivatives (5)

To a solution of 10-(3-phenyl oxopropen -1-yl) phenoxazie (3) (0.313g, 0.001mole) in ethanol (20 ml), was added phenyl hydrazine (0.830g, 0.007 mole) and few drops of piperidine. The mixture was refluxed for 5hrs. On cooling, a gumy deposit separated out.

This was crystallized from ethanol to give (5a-5i). Table (3) shows the physical data of compounds (5a-5i).

Pt IV: 10- (-isoxazolin -3- yl) phenoxazine derivatives (6)

A solution of (0.313g, 0.001 mole) of (3) and (0.07 g ,0.001 mole) of hydroxyl amine hydrochloride in 1% ethanolic sodium hydroxide solution was refluxed for 6hr. On cooling, the product separated out, crystallized from ethanol. FTIR of this compound showed bands at (1570-1595) cm^{-1} (C =Cstr.) and (1588-1618) cm^{-1} (C=N str.). The physical data are described in Table (4).

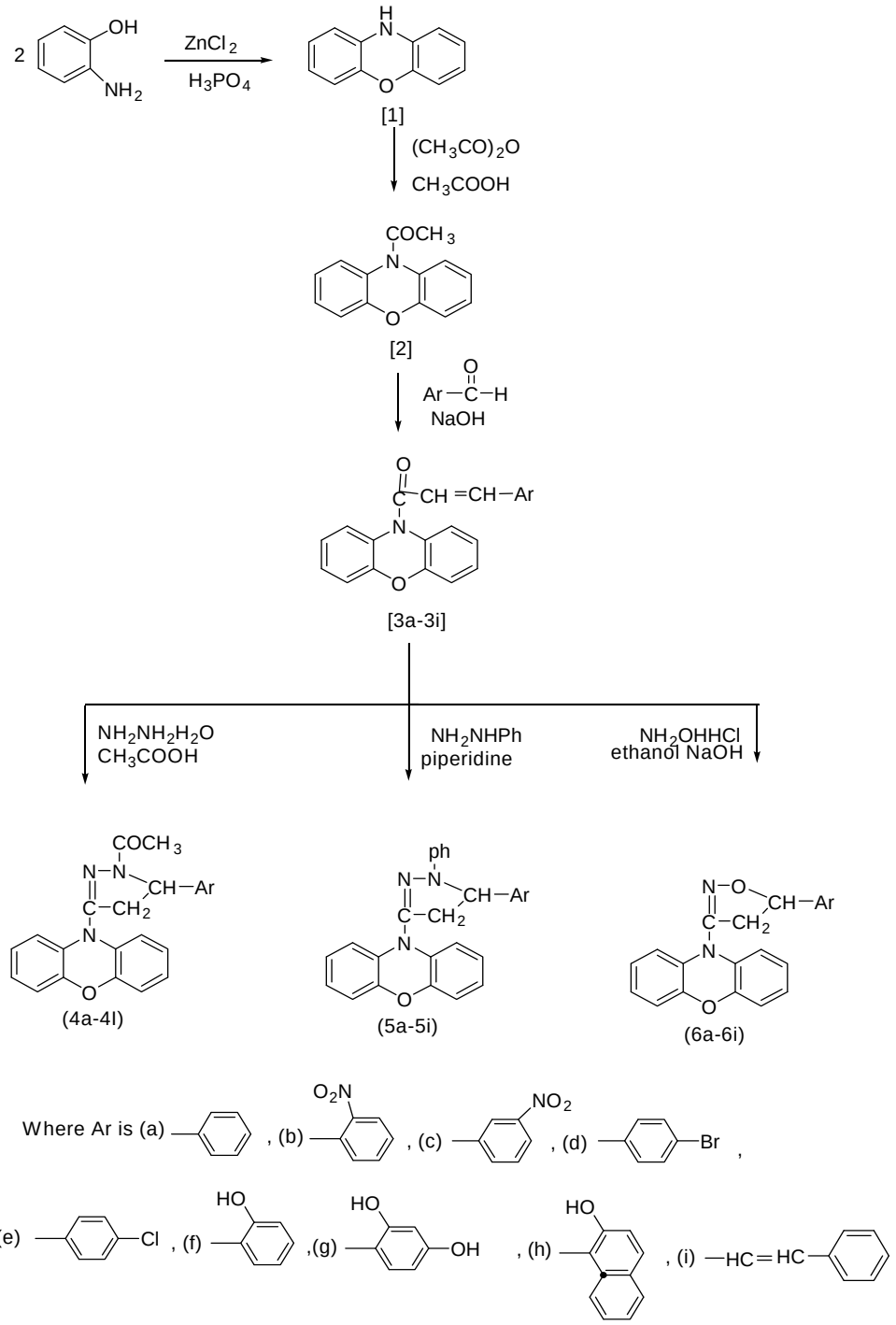
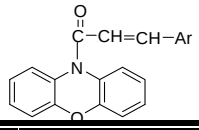

2
OH
NH2
ZnCl2
H3PO4
H
N
O
[1]
(CH3CO)2O
CH3COOH
COCH3
[2]
Ar—C—H
O
NaOH
CH =CH—Ar
[3a-3i]
NH2NH2H2O
CH3COOH
NH2NHPh
piperidine
NH2OHHCl
ethanol NaOH
CH—Ar
CH2
ph
(4a-4l)
(5a-5i)
(6a-6i)
Where Ar is (a)
, (b)
O2N
, (c)
NO2
, (d)
Br
(e)
Cl
, (f)
HO
,(g)
OH
, (h)
, (i)
HC= HC

Table 1: Physical properties of compounds (3a-3i).



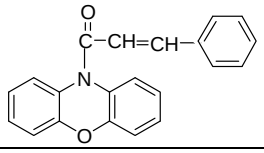
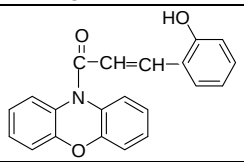
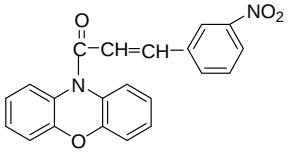
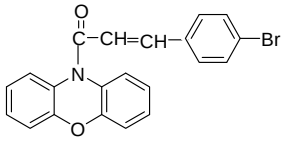
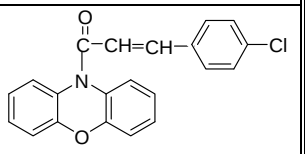
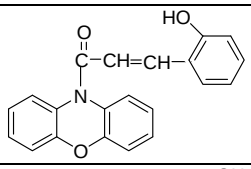
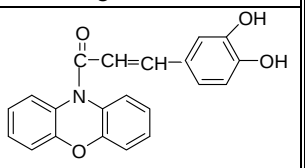
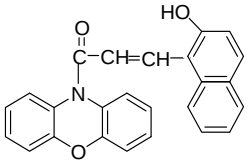
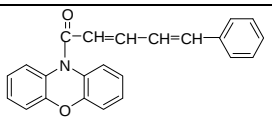
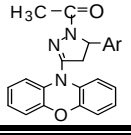
Compd. No.	Sientific name	M.wt	m.p C^0	% Yield	Color of crystal	Chemical structure
3a	10-(3-phenyloxoprop -2-en-1-yl) phenoxazine	313	101	80	Olive green	
3b	10-[3-(2-nitrophenyl) oxoprop -2-en-1-yl] phenoxazine	358	137	45	Brown	
3c	10-[3-(3-nitrophenyl) oxoprop -2-en-1-yl] phenoxazine	358	114	42	Brown	
3d	10-[3-(4-bromophenyl) oxoprop -2-en-1-yl] phenoxazine	392	144	31	Brown	
3e	10-[3-(4-clorophenyl) oxoprop -2-en-1-yl] phenoxazine	347.5	146	45	Brown	
3f	10-[3-(2-hydroxyphenyl) oxoprop -2-en-1-yl] phenoxazine	329	108	24	Olive green	
3g	10-[3-(3,4-dihydroxyphenyl) oxoprop -2-en-1-yl] phenoxazine	345	142	23	Light green	
3h	10-[3-(2-hydroxy-1-naphthyl) oxoprop -2-en-1-yl] phenoxazine	379	96	64	Brown	
3i	10-[5-phenyl)oxoprop -2,4-dien-1-yl] phenoxazine	339	115-118	92	Brow	

Table 2: Physical properties of compounds (4a-4i).



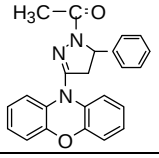
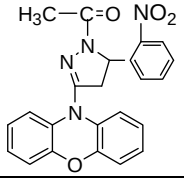
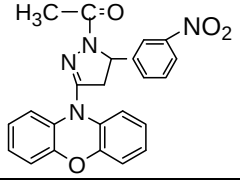
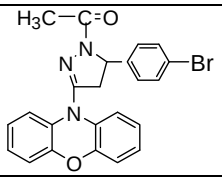
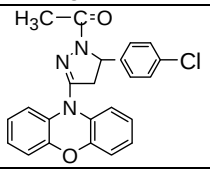
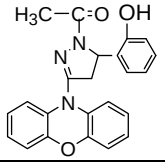
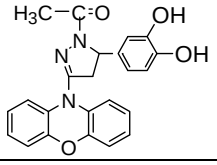
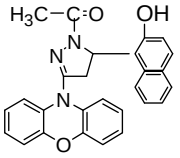
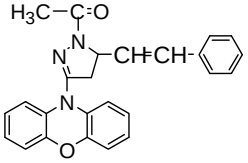
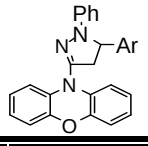
Compd. No.	Sientific name	M.wt	m.p C^0	% Yield	Color of crystal	Chemical structure
4a	10-(1-acetyl-5-phenylpyrazolin-3-yl) phenoxazine	360	122	36	Light Brown	
4b	10-[1-acetyl-5-(2-nitrophenyl)pyrazolin-3-yl] phenoxazine	414	124	36	Grey	
4c	10-[1-acetyl-5-(3-nitrophenyl)pyrazolin-3-yl] phenoxazine	414	103	70	Brown	
4d	10-[1-acetyl-5-(4-bromophenyl)pyrazolin-3-yl] phenoxazine	448	124	67	Light Brown	
4e	10-[1-acetyl-5-(4-chlorophenyl)pyrazolin-3-yl] phenoxazine	403.5	140	42	Off White	
4f	10-[1-acetyl-5-(2-hydroxyphenyl)pyrazolin-3-yl] phenoxazine	385	123	24	Olive Green	
4g	10-[1-acetyl-5-(3,4-dihydroxyhpenyl)pyrazolin-3-yl] phenoxazine	401	108 dec.	79	Light Brown	
4h	10-[1-acetyl-5-(2-hydroxynaphthyl)pyrazolin-3-yl] phenoxazine	435	120	33	Off White	
4i	10-[1-acetyl-5-styrenyl)pyrazolin-3-yl] phenoxazine	395	140	48	Golden yellow	

Table 3: Physical properties of compounds (5a-5i).



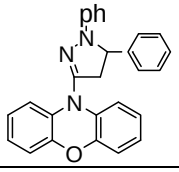
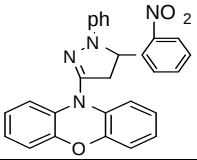
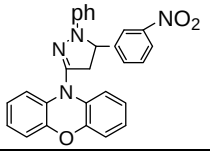
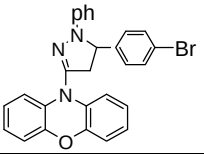
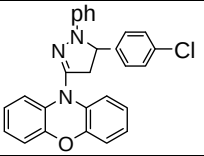
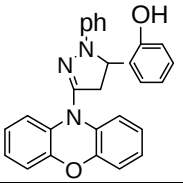
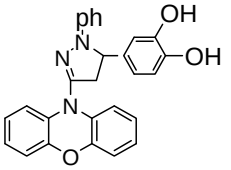
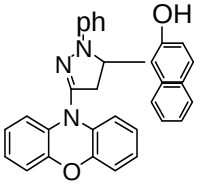
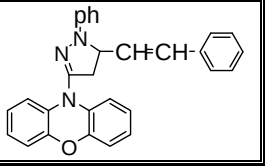
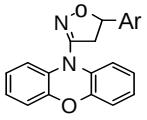
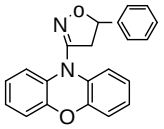
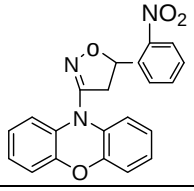
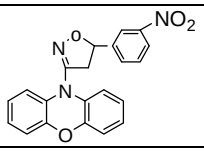
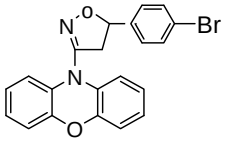
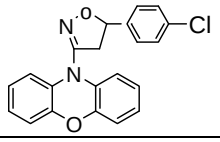
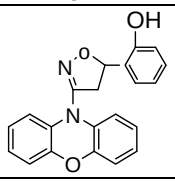
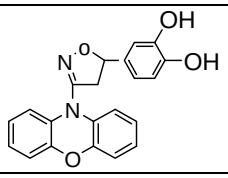
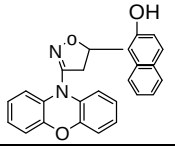
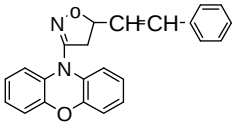
Compd. No.	Sientific name	M.wt	m.p C^0	% Yield	Color of crystal	Chemical structure
5a	10-(1,5-diphenylpyrazolin-3-yl) phenoxazine	403	98	32	Off white	
5b	10-[1-phenyl-5-(2-nitrophenyl)pyrazolin-3-yl] phenoxazine	448	128	51	Brown	
5c	10-[1- phenyl -5-(3-nitrophenyl)pyrazolin-3-yl] phenoxazine	448	127	70	Pale yellow	
5d	10-[1- phenyl -5-(4-bromophenyl)pyrazolin-3-yl] phenoxazine	442	121	47	White	
5e	10-[1- phenyl -5-(4-chlorophenyl)pyrazolin-3-yl] phenoxazine	407.5	111	57	Brown	
5f	10-[1- phenyl -5-(2-hydroxyphenyl)pyrazolin-3-yl] phenoxazine	419	130	37	Off white	
5g	10-[1- phenyl -5-(3,4-dihydroxyhpenyl)pyrazolin-3-yl] phenoxazine	435	164	51	Light Brown	
5h	10-[1- phenyl -5-(2-hydroxynaphthyl)pyrazolin-3-yl] phenoxazine	469	117	25	Dark Brown	
5i	10-[1- phenyl -5-styrenyl)pyrazolin-3-yl] phenoxazine	429	134	74	Light green	

Table 4: Physical properties of compounds (6a-6i).



Compd. No.	Sientific name	M.wt	m.p C^0	% Yield	Color of crystal	Chemical structure
6a	10-(5-phenylisoxazolin-3-yl) phenoxazine	328	84	23	Deep Brown	
6b	10-[5-(2-nitrophenyl) isoxazolin-3-yl] phenoxazine	373	117	39	Light Brown	
6c	10-[5-(3-nitrophenyl) isoxazolin-3-yl] phenoxazine	373	102	11	Off white	
6d	10-[5-(4-bromophenyl) isoxazolin-3-yl] phenoxazine	406.9	137	62	Grey	
6e	10-[5-(4-chlorophenyl) isoxazolin-3-yl] phenoxazine	362.5	149	50	Brown	
6f	10-[5-(2-hydroxyphenyl) isoxazolin-3-yl] phenoxazine	344	133	36	Off white	
6g	10-[5-(3,4-dihydroxyhpenyl) isoxazolin-3-yl] phenoxazine	360	165 Dec.	49	Grey	
6h	10-[5-(2-hydroxynaphthyl) isoxazolin-3-yl] phenoxazine	396	144	80	Off white	
6i	10-[5-styrenyl) isoxazolin-3-yl] phenoxazine	353	129	60	White	

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